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13. ABSTRACT *(Maximum 200 Words)*

Segregation analyses of familial prostate cancer have provided evidence for the existence of dominantly-acting prostate cancer susceptibility alleles, with such genes being estimated to be responsible for about nine percent of all cases of prostate cancer in the U.S. These findings provided the basis for our genome wide scan for linkage in hereditary prostate cancer (HPC) families, leading to the identification of the *HPC1* locus at 1q24-25 as the first reported linkage in prostate cancer (Smith et al., *Science* 274:1371, 1996). Since this finding multiple other HPC loci have been identified, including our finding of the *HPCX* locus at Xq27-28 (Xu et al. *Nat. Gen.* 20:175, 1998). These results emphasize the genetic heterogeneity that characterizes HPC. To increase the power of our family collection in an effort to deal with this heterogeneity, we have collected an additional 57 HPC families, each having over 4 individuals affected with prostate cancer. We have carried out genotypic analysis of our complete collection of 175 pedigrees at a series of putative HPC loci, including loci implicated by other research groups on chromosomes 1, 8, 20 and 17 (HPC2). While little evidence of linkage was found at this latter locus, novel loci were identified on both chromosomes 1 and 8. By accumulating linkage data on our complete set of HPC families, we are able to begin to understand and evaluate genetic heterogeneity of HPC, as well as to provide critical positional information for gene mapping and identification studies. Such studies are prerequisite to the development of genetic tests for determination of prostate cancer susceptibility.

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Introduction

In spite of the magnitude of the problem which prostate cancer presents, our understanding of the molecular mechanisms underlying prostatic carcinogenesis remains elusive. It is clear from the recent progress made in colorectal, renal and breast cancer that analysis of familial forms of common human neoplasms can yield tremendous insight into the specific genetic mechanisms in both hereditary and sporadic forms of such cancers. Hereditary factors are estimated to be responsible for about nine percent of all cases of prostate cancer in the U.S. Segregation analysis of familial prostate cancer has supported an autosomal dominant mode of inheritance of prostate cancer susceptibility alleles with some evidence for heterogeneity. These findings provided the basis for a genome wide scan for linkage in multiplex prostate cancer families. This analysis implicated 1q24-25 as being the most likely region of the genome to contain a major prostate cancer susceptibility gene (HPC1). Interestingly, this evidence for linkage was provided almost exclusively by large families (5 or more first degree relatives affected/family) with an early average age of diagnosis (<65 years). However, there was significant evidence for locus heterogeneity and a series of other loci also showed evidence of linkage, albeit to a lesser extent than HPC1. *It is the goal of the research proposed herein to further analyze these other regions for evidence of linkage to prostate cancer susceptibility.* To detect these potential linkages, 57 additional families, each containing at least five affected members and over half having an average age of diagnosis under 65, have been collected for these studies, as deemed necessary from simulation analyses. Genotypic data for these families in the regions of interest have been analyzed using both parametric and non-parametric methods, including conditional analyses and two locus models to test for gene-gene interactions. These studies provide the basis for positional cloning efforts to identify and characterize prostate cancer susceptibility genes.

Body

Progress report for Activities During Months 1-24

Listed below is a summary of the research objectives as described in the approved Statement of Work as it applies to the 30 months of the funding period, along with the accomplishments pertaining to these objectives.

Task 1) ***Ascertain 57 additional families with at least 5 members with prostate cancer (months 1-30).***

Accomplishments related to Task 1: Within the 30 months of Phase I, we ascertained 57 families as proposed in our specific aims. We contacted each living family member to obtain informed consent and blood DNA. Blood or tissue samples have been obtained from 268 individuals in these new families. Table I summarizes the characteristics of our complete family collection of 171 HPC families, each having at least 3 first degree relatives with prostate cancer. Fifty-three % of these families have 5 or more first degree relatives affected.

Table I HPC Family Collection

	All Families (n=175)	Families with 5 or more Affected Men (n=96)
# Affected	857	502
Average # Affected per family	4.9	5.3
# Blood DNAs	1030	657
Average Age of Diagnosis per family	64.2	63.8
# Unaffected Males	275	179
# Females	379	273
# Affected with Age of Dx < 65	371	243

Task 2) ***Genotype the new and current sets of families for highly polymorphic markers in the chromosomal regions for which we have preliminary evidence of linkage (months 1-30).***

Accomplishments related to Task 2: Genotypes have been generated for over 1020 individuals in the existing 175 families for the following sets of markers: Xq27-28, 40 markers; 8p, 20 markers; 13q, 25 markers; 1q42-43, 6 markers; 1p36, 6 markers; 16 markers located in between 1p36, 1q24-25 and 1q42-43; 17p13, 6 markers; 20q, 16 markers for a total of 135 loci. DNA has been prepared from 250 individuals in the 57 new families and a majority of these markers have been analyzed in this dataset.

Task 3) ***Perform genetic linkage analysis on the existing 102 and 57 new HPC families (months 3-30).***

Accomplishments related to Task 3: Analysis of marker data for chromosomes 1, 17 (HPC2), 20, and a novel locus on chromosome 8 are summarized here, and presented in more detail in the attached papers and manuscripts.

Summary Results for Chromosome 1

1. Genotyping of markers spanning chromosome 1, with emphasis on *CaPB* at *1p36*, *PCaP* at *1q42-43*, and *HPC1* at *1q24-25*, reveals significant evidence of linkage only at *HPC1* in this collection of 159 HPC families. (see attached reprint, Xu et al., *Hum. Genetics* 108:335-345, 2001).

Summary results for Chromosome 20

1. Genotyping of markers spanning 95 cM on chromosome 20, with emphasis on *HPC20* at *20q13* provided positive evidence of linkage from 20pter to 20q11 with the highest non-parametric linkage score for the complete dataset of 1.02 (p=0.15) being observed at D20S195 at 20q11. (see attached reprint by Zheng et al. *Hum. Genetics* in press, 2001).

Summary Results for HPC2/ELAC2 at 17p11

1. Using a combined mutation, linkage and association approach, we were unable to obtain confirming evidence for an important role for HPC2 in prostate cancer susceptibility (see attached reprint, Xu et al., *AJHG* 68:901-911, 2001).

Summary Results for Chromosome 8

Background for studying 8p

1. An elevated lod score was observed in our initial analysis of 66 HPC families. The lod scores was 1.24 at D8S550.
2. Many LOH have demonstrated frequent inactivation of one of more prostate tumor suppressor genes on 8p, but these genes remain unidentified.
3. A French biotech company (GENSET), led by Daniel Cohen, identified a putative prostate cancer susceptibility gene (PG1) at 8p23, described in US Patent #5,945,522 (8/31/99).

Purpose

a. To investigate the linkage between prostate cancer susceptibility gene and 8p markers in 159 HPC families
b. To examine linkage to PG1 using both family-based association method in 159 HPC families and population-based association method in 159 HPC cases, 47 sporadic cases and 91 controls. Since parental genotype data is usually unavailable in prostate cancer sample, we used the Reconstruction-combined Transmission Disequilibrium Test (RC-TDT) method for the family-based association test. The RC-TDT systematically reconstructs parental genotype based on offspring data and combined with sib-pair TDT. This approach has better power to detect association and linkage.

Results

1. Evidence for linkage was observed in the 159 HPC families. The peak lod score assuming heterogeneity was 1.91 at marker 17 (Table 3). The peak NPL score was 2.68 (p=0.004) at the same marker. These values meet the Lander-Kruglyak criteria for suggestive linkage (Nature Genetics 11:241-247, 1995).
2. The evidence for linkage in 11 Ashkenazi families was stronger. The proportion of families linked to this region ranged from 60% to 96% (Table 4)
3. There was no evidence for association between PG1 and prostate cancer using RC-TDT method in 159 HPC families. However, there were several markers at the peak linkage region with marginally significant p-values (Table 5)
4. There was no evidence for association between PG1 and prostate cancer using case-control analysis. There was no statistical difference in the SNPs allele frequency between 159 HPC cases, 47 sporadic cases, and 91 unaffected controls (Table 6). There was also no statistical difference in the haplotype frequency between cases and controls (Table 7).
5. These results indicate the presence of an HPC gene on chromosome 8, but effectively exclude PG1 as being this gene.

Table 3. Multipoint Linkage Results at 8p Region in 159 HPC Families

	Multipoint		NPL	
	hlod	alpha	Z-score	P-value
Marker1	0.24	0.06	0.41	.33
Marker2	0.51	0.08	1.12	.13
Marker3	0.59	0.08	1.36	.08
Marker4	0.25	0.05	1.15	.12
Marker5	0.55	0.08	1.49	.07
PG1-a	0.60	0.08	1.51	.07
PG1-b	0.60	0.08	1.51	.07
PG1-c	0.60	0.08	1.54	.06
PG1-d	0.60	0.08	1.53	.06
PG1-e	0.61	0.08	1.55	.06
Marker11	0.68	0.08	1.88	.03
Marker12	1.19	0.12	2.56	.006
Marker13	1.19	0.12	2.50	.007
Marker14	1.14	0.12	2.28	.01
Marker15	1.26	0.12	2.33	.01
Marker16	1.25	0.12	2.39	.009
Marker17	1.91	0.15	2.68	.004
Marker18	1.10	0.12	2.48	.008
Marker19	1.23	0.12	2.68	.004
Marker20	0.47	0.08	1.30	.09

Table 4. Multipoint Linkage Results at 8p Region in Three Race/Ethnic Groups

marker	African American		Ashkanazi		Caucasian	
	alpha	HLOD	alpha	HLOD	alpha	HLOD
Marker1	0.00	0.00	0.45	0.44	0.07	0.23
Marker2	0.00	0.00	0.45	0.49	0.10	0.64
Marker3	0.00	0.00	0.48	0.59	0.10	0.71
Marker4	0.00	0.00	0.46	0.59	0.06	0.30
Marker5	0.00	0.00	0.44	0.56	0.09	0.66
PG1-a	0.00	0.00	0.46	0.54	0.09	0.64
PG1-b	0.00	0.00	0.47	0.55	0.09	0.64
PG1-c	0.00	0.00	0.47	0.56	0.09	0.64
PG1-d	0.00	0.00	0.48	0.57	0.09	0.64
PG1-e	0.00	0.00	0.49	0.58	0.09	0.64
Marker11	0.00	0.00	0.59	0.91	0.08	0.63
Marker12	0.00	0.00	0.60	1.00	0.12	1.12
Marker13	0.00	0.00	0.60	1.01	0.12	1.12
Marker14	0.00	0.00	0.60	1.03	0.12	1.06
Marker15	0.00	0.00	0.61	1.04	0.13	1.17
Marker16	0.00	0.00	0.61	1.05	0.13	1.16
Marker17	0.00	0.00	0.62	1.06	0.15	1.82
Marker18	0.00	0.00	0.66	1.10	0.12	0.97
Marker19	0.20	0.49	0.72	1.14	0.09	0.48
Marker20	0.27	0.64	0.96	1.24	0.02	0.01

Table 5. Reconstructed-combined TDT at 8p Region

	Allele	P_exact	P_Z
Marker1	9	0.008	0.006
Marker2	6	0.05	0.04
Marker3	Any	n.s.	n.s.
Marker4	Any	n.s.	n.s.
Marker5	Any	n.s.	n.s.
PG1-a	Any	n.s.	n.s.
PG1-b	G	n.s.	n.s.
PG1-c	C	n.s.	n.s.
PG1-d	Any	n.s.	n.s.
PG1-e	T	n.s.	n.s.
Marker11	5	0.03	0.02
Marker12	Any	n.s.	n.s.
Marker13	Any	n.s.	n.s.
Marker14	4	0.04	0.03
Marker15	8	0.04	0.02
Marker16	11	0.06	0.04
Marker17	Any	n.s.	n.s.
Marker18	7	0.03	0.03
Marker19	14	0.03	0.03
Marker20	Any	n.s.	n.s.

Table 6. Allele frequencies of SNPs in PG1

	# of sample	PG1-b allele 'G'	PG1-e allele 'T'
Controls	91	0.25	0.21
All cases	218	0.31	0.24
Sporadic cases	47	0.34	0.27
HPC cases	171	0.28	0.23
HPC cases, lod >0	45	0.3	0.24

The difference in the allele frequencies is not statistically significant

Table 7. Haplotype frequencies of SNPs in PG1

	# of sample	PG1-b and PG1-e ('T-G')
Controls	91	0.185
All cases	218	0.208
Sporadic cases	47	0.207
HPC cases	171	0.209
HPC cases, lod >0	45	0.22

The difference in the haplotype frequencies is not statistically significa

Key Research Accomplishments

- ascertainment of 57 new HPC families, with an average of 4.8 prostate cases per family
- collection of blood samples form 268 individuals in these families, and the preparation of DNA and lymphoblastoid cell lines from these individuals
- genotyping of 135 marker loci on our existing family collection and a subset of the newly ascertained families
- two-point and multipoint linkage analyses of these data have been completed for these markers
- little evidence is found to implicate the HPC2 gene at 17p11 in prostate cancer susceptibility.
- novel loci have been implicated on 8p, and 1p.
- confirmatory data has been obtained to support an HPC gene at 20q.

Reportable Outcomes

- manuscripts
    - Xu et al. *Nat. Gen.* 20:175, 1998 Evidence for a Prostate Cancer Susceptibility Locus on the X Chromosome.
    - Xu et al. *Am J Hum Genet* Mar;66 (3):945-57, 2000. Combined Analysis of Hereditary Prostate Cancer Linkage to 1q24-25: Results from 772 Hereditary Prostate Cancer Families from the International Consortium for Prostate Cancer Genetics.
    - Jianfeng Xu, Siqun L. Zheng, Bao-li Chang, Jeffrey R. Smith, John D. Carpten, O. Colin Stine, Sarah D. Isaacs, Kathy Wiley, Lauren Henning, Charles Ewing, Piroska Bujnovszky, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Linkage of prostate cancer susceptibility loci to chromosome 1. *Hum Genetics* 108:335-345, 2001.
    - Jianfeng Xu, Siqun L. Zheng, John D. Carpten, Nina N. Nupponen, Christiane Robbins, Juanita Mestre, Tracy Moses, Dennis Faith, Brian Kelly, Sarah D. Isaacs, Kathy Wiley, Bao-li Chang, Joan Bailey-Wilson, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Evaluation of linkage and association of HPC2/ELAC2 in familial and unrelated prostate cancer patients. *American Journal of Human Genetics* 68:901-911, 2001.
    - Siqun L. Zheng, Jianfeng Xu, Sarah D. Isaacs, Kathy Wiley, Bao-li Chang, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Evidence of linkage to chromosome 20 in 159 hereditary prostate cancer families. *Hum Genetics*, In press, 2001.
    - Jianfeng Xu, Siqun L. Zheng, Bao-li Chang, Jeffrey R. Smith, John D. Carpten, O. Colin Stine, Sarah D. Isaacs, Kathy Wiley, Lauren Henning, Charles Ewing, Piroska Bujnovszky, Patrick C. Walsh, Jeffrey M. Trent, Deborah A. Meyers, William B. Isaacs. Identification of a novel HPC locus on chromosome 8. Submitted.

Conclusions

A cohort of 57 new hereditary prostate cancer families containing 274 affected men has been ascertained, and blood samples collected from 268 family members. These families combined with our previous collection provide a unique resource of 175 HPC families, highly informative for linkage analysis. Genotyping has been carried out on these families, and linkage analysis of these data performed. These analyses have led to the demonstration that, of all putative HPC loci identified to date, the three most important in our family collection are *HPC1*, *HPCX* and a novel locus on chromosome 8 (which is not PG1). These findings provide the basis for extended efforts to identify prostate cancer susceptibility genes and will greatly increase our ability to understand and characterize the genetic heterogeneity of hereditary prostate cancer. It is critical to understand this aspect of HPC if we are to develop meaningful genetic tests to identify individuals at high risk of developing this disease.

Am. J. Hum. Genet. 68:901–911, 2001

Evaluation of Linkage and Association of HPC2/ELAC2 in Patients with Familial or Sporadic Prostate Cancer

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To investigate the relationship between HPC2/ELAC2 and prostate cancer risk, we performed the following analyses: (1) a linkage study of six markers in and around the HPC2/ELAC2 gene at 17p11 in 159 pedigrees with hereditary prostate cancer (HPC); (2) a mutation-screening analysis of all coding exons of the gene in 93 probands with HPC; (3) family-based and population-based association study of common HPC2/ELAC2 missense variants in 159 probands with HPC, 249 patients with sporadic prostate cancer, and 222 unaffected male control subjects. No evidence for linkage was found in the total sample, nor in any subset of pedigrees based on characteristics that included age at onset, number of affected members, male-to-male disease transmission, or race. Furthermore, only the two previously reported missense changes (Ser217Leu and Ala541Thr) were identified by mutational analysis of all HPC2/ELAC exons in 93 probands with HPC. In association analyses, family-based tests did not reveal excess transmission of the Leu217 and/or Thr541 alleles to affected offspring, and population-based tests failed to reveal any statistically significant difference in the allele frequencies of the two polymorphisms between patients with prostate cancer and control subjects. The results of this study lead us to reject the three alternative hypotheses of (1) a highly penetrant, major prostate cancer–susceptibility gene at 17p11, (2) the allelic variants Leu217 or Thr541 of HPC2/ELAC2 as high-penetrance mutations, and (3) the variants Leu217 or Thr541 as low-penetrance, risk-modifying alleles. However, we did observe a trend of higher Leu217 homozygous carrier rates in patients than in control subjects. Considering the impact of genetic heterogeneity, phenocopies, and incomplete penetrance on the linkage and association studies of prostate cancer and on the power to detect linkage and association in our study sample, our results cannot rule out the possibility of a highly penetrant prostate cancer gene at this locus that only segregates in a small number of pedigrees. Nor can we rule out a prostate cancer–modifier gene that confers a lower-than-reported risk. Additional larger studies are needed to more fully evaluate the role of this gene in prostate cancer risk.

Introduction

Using a genomewide screen together with positional cloning, Tavtigian et al. (2001) identified the HPC2/ELAC2 gene (MIM 605367) on chromosome 17 as a prostate cancer (MIM 176807) susceptibility gene in large, high-risk Utah pedigrees. A genomewide screen in eight Utah pedigrees provided suggestive evidence for linkage at 17p11 near marker D17S520, and fine-mapping studies using dense markers in the region in a larger set of pedigrees (total of 33) provided significant evidence for linkage, with a maximum two-point LOD score of 4.5 at D17S1289. The evidence for linkage in an additional 94 pedigrees was positive but much weaker, with a peak LOD of 0.44 in this region. Sequence analysis of HPC2/ELAC2 identified four sequence variants, including a rare frameshift, and three missense changes, two of which were common in the study population. These latter two polymorphisms result in a Ser-to-Leu change at amino acid 217, and an Ala-to-Thr change at amino acid 541. These two polymorphisms were reported to segregate with prostate cancer in two high-risk pedigrees. In addition, the two polymorphisms were found to be associated with the diagnosis of prostate cancer, by comparing the carrier rates of Leu217 and/or Thr541 among patients with heredi-

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tary prostate cancer (HPC), unaffected members of pedigrees with HPC, and unaffected men who had married into the pedigrees. Specifically, the overall allele frequency of Leu217 was 30%, and the frequency of Leu217 homozygotes was higher in patients with HPC (57 [13.3%]/429) than in either unaffected members of pedigrees with HPC (220 [9.3%]/2371; $P = .013$) or in unaffected men who had married into the pedigrees (9/148 [6.1%], $P = .026$). The overall allele frequency of Thr541 was 4% and its carrier rates were higher in patients with HPC (42 [9.8%]/429) than in unaffected men who had married into the pedigrees (5 [3.4%]/148; $P = .022$). There is strong linkage disequilibrium (LD) between the two polymorphisms, even though they are ~15 kb apart. All of the Thr541 variants are observed on Leu217 chromosomes (Tavtigian et al. 2001).

The finding of the association between the two polymorphisms in HPC2/ELAC2 and prostate cancer risk was examined in an independent study of 359 incident prostate cancer case subjects unselected for family history and 266 male control subjects without prostate cancer (Rebbeck et al. 2000). The frequencies of the Leu217 allele was 30.8%, 31.5%, and 31.6%, in the 359 case subjects, 258 age- and race-matched control subjects, and 383 total control subjects, respectively. There was no significant difference of the Leu217 carrier rates in the case subjects (52.1%) and in the control subjects (53.1%). The frequencies of Thr541 allele were 3.8%, 1.8%, and 2.9% in the 359 case subjects, 258 age- and race-matched control subjects, and all 383 control subjects (matched and unmatched), respectively. The Thr541 carrier rate was reported to be significantly higher in the case subjects (7.5%) than in the matched control subjects (3.5%), with an odds ratio (OR) of 2.37 (95% confidence interval [CI] 1.06–5.29), with the Leu217/Thr541 variant being estimated to account for ~5% of prostate cancer case subjects in the general population. Interestingly, the Thr541 carrier rate in case subjects was not significantly higher than the frequency observed for the complete control group (5.7%). Rebbeck et al. also reported that the Thr541 allele was only observed on the background of Leu217.

To examine the above findings of linkage and association between the HPC2/ELAC2 and prostate cancer risk, we performed linkage and mutational analyses in families with HPC and association studies in two data sets. We genotyped four microsatellite markers surrounding the HPC2/ELAC2 gene and the Leu217 and Thr541 polymorphisms within the gene in 159 families with hereditary prostate cancer. We have also genotyped the two polymorphisms in 249 sporadic prostate cancer case subjects, and 211 non–prostate cancer male control subjects. In addition, we performed mutational analysis on all HPC2/ELAC exons by heteroduplex analysis and direct sequencing of 93 patient DNA samples.

With these data, we can test the following alternative hypotheses. (1) If the HPC2/ELAC2 is a major, high-penetrance gene for prostate cancer, we would expect to observe significant linkage at 17p11 in the 159 total families with HPC and/or mutations in the gene segregating with disease phenotype. (2) If the variants Leu217 or Thr541 of the gene HPC2/ELAC2 were high-penetrance mutations, we would expect to observe significant linkage, in the subset of families that carry the Leu217 and/or Thr514 alleles, and over-transmission of the Leu217 and/or Thr541 alleles in these families. (3) If the variants Leu217 or Thr541 are high-prevalence but low-penetrance modifier alleles, we would expect to observe higher frequency of the Leu217 and/or Thr514 allele carrier in sporadic case subjects, compared with unaffected control subjects.

Families and Methods

Ascertainment of Families

All 159 families with HPC were ascertained and studied at the Brady Urology Institute at Johns Hopkins Hospital. Families were ascertained from three sources. Sixty-eight families were ascertained through referrals generated in response to a letter by one of us (PCW) to 8,000 urologists throughout the United States. The second source, from which 37 families were identified, was family-history records of patients seen at Johns Hopkins Hospital for treatment of prostate cancer. The remaining families (54) came from the respondents to articles, which appeared in various lay publications, describing our studies of families with HPC. Prostate cancer diagnosis was verified by medical records for each affected man studied. Age at diagnosis of prostate cancer was confirmed either through medical records or through two other independent sources. The mean age at diagnosis was 64.3 for the case subjects in these families; 84% of the families are white, and 8.8% are black.

All sporadic prostate cancer case subjects were recruited from among patients who underwent treatment for prostate cancer at the John Hopkins Hospital. The diagnosis of prostate cancer for all these subjects was confirmed by pathology reports. Preoperative prostate-specific antigen (PSA) levels, Gleason score, and pathological stages were available for 92, 244, and 245 of the 249 sporadic case subjects, respectively. Mean age at diagnosis for these case subjects was 58.6. Family histories were not available. More than 93% of the case subjects are white, and 3.2% are black.

Two hundred and twenty-two control subjects were selected from among men who participated in screening programs for prostate cancer. After excluding those who had abnormal results of a digital rectal examination (DRE) or abnormal PSA levels ($\geq$4 ng/ml), 211 were

eligible for the study. The mean age at examination was 58 years. More than 86% of the eligible control subjects are white and 7.1% are black. About 5.6% of the eligible control subjects have a brother(s) or father affected with prostate cancer. The affection status of relatives was obtained by interview of the probands.

Marker Genotyping

Four microsatellite markers surrounding the HPC2/ELAC2 gene were genotyped in 159 HPC families. These markers were selected from Marshfield Comprehensive Human Genetic Maps (Broman et al. 1998) and cover ~18 cM from 17p13 to 17q1. Multiplex PCR using fluorescently labeled primers (either fam, hex, or ned) was performed, and the resulting PCR fragments were separated by means of capillary electrophoresis using an ABI 3700 DNA Analyzer (Applied Biosystems). The genotypes were scored using ABI Genotyper software. A modified version of the program Linkage Designer was used to bin the alleles and check inheritance. The output from Linkage Designer was then analyzed further for any inconsistencies by use of the program LINKAGE (Lathrop et al. 1984; Cottingham et al. 1993) without disease-phenotype information. Marker allele frequencies were estimated from the independent individuals in the data set (i.e., genetically unrelated individuals based on all the available information).

Two single-nucleotide polymorphisms (SNPs) in the HPC2/ELAC2 gene were genotyped for all subjects using PCR and restriction enzyme digestion, as described by Rebbeck et al. (2000), with the following modifications: for the region containing the Ser217Leu variant, PCR was performed in a 10-μl volume consisting of 30 ng genomic DNA, 0.2 μM each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 20 mM Tris-HCl, 50 mM KCl and 0.5 U *Taq* polymerase (Life Technologies). The primers were m5A (5′-CATTCCCATGTATGAACGTCT-3′) and m5Q (5′-AGGAAACAGCTATGACCATCTACAAGCATTA-CAAGGCAGAG-3′). These primers amplified a 276-bp fragment. PCR cycling conditions were as follows: 95°C for 3 min, followed by 28 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 20 s, with a final extension of 72°C for 2 min; 5 μl of PCR products were digested with *Taq*αI (New England Biolabs) at 65°C for 2 hours. Genotypes were read on 2% agarose gels. Among Ser/Ser individuals, 172- and 104-bp products were observed, whereas Leu/Leu produced an uncut 276-bp band. For the region containing the Ala541Thr variant, PCR was performed as with the Ser217Leu variant. The primers were m15A (5′-CCAGCCTTTGTGTAAGTCTAC-3′) and m15P (5′-TCTGGGCAAGTTTGGAAGC-3′). A 495-bp fragment was amplified. PCR cycling conditions were the same as for Ser217Leu, except that the annealing temperature was 57°C; 5 μl of PCR products were then digested with *Fnu*4HI (New England Biolabs) at 37°C for 2 h, and the fragments were separated on 2% agarose gels. Among Thr/Thr individuals, 162-bp products were observed; for Ala/Ala, 110-bp products were observed. Genotyping of the two SNPs in 159 HPC probands was performed in three independent laboratories (Wake Forest, National Human Genome Research Institute, and Johns Hopkins University) as a quality-control measure. All the genotyping results were identical.

Genomic Mutational Analysis

For HPC2/ELAC2 exons, PCR was performed in 50-μl reactions consisting of 20 ng genomic DNA, 10 mM dNTPs, 10 × PCR Buffer (Gibco BRL), 4.5 mM $MgCl_2$, 0.5 U Platinum™ *Taq* DNA polymerase (Gibco BRL), 0.5 U Ampli*Taq* Gold™ (Applied Biosystems), and 10 pmol of each forward and reverse primer (Gibco BRL). PCR cycles consisted of 95°C for 14 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 5 min. PCR products were denatured for 3 min at 95°C and then were reannealed gradually over 30 min using a 95°C to 65°C temperature gradient. The optimal melting temperature for each PCR amplicon was obtained by analysis of wild-type sequence, using an algorithm at the Stanford Denaturing High-Performance Liquid Chromatography (DHPLC) Web site.

DHPLC

DHPLC heteroduplex analysis was performed using automated HPLC instrumentation equipped with an analytical 2.1 × 75–mm Eclipse dsDNA column (Agilent Technologies). The analytical gradient was composed of Buffer A (100 mM triethylammonium acetate and 0.10 mM EDTA) and Buffer B (100 mM triethylammonium acetate, 0.10 mM EDTA, and 25% acetonitrile) with a flow rate of 0.4 ml/min. The injection volume of each PCR sample was 4 μl. The analysis time for each sample was 10 min, including a column wash and an equilibration step.

Sequencing Analysis

PCR products were purified using the Qiagen PCR purification kit (Qiagen) with the Qiagen BIOROBOT 9600 dual vacuum system. One-half–volume sequencing reactions were prepared in a 96-well format using the 3700 Big Dye™ Terminator Chemistry (Applied Biosystems) as follows: 6 μl of purified PCR product, 4 μl Big Dye Terminator reagent, and 1 μl of 5 pmol/μl of primer. Exons 7, 11, and 17 were sequenced using M13 forward and reverse primers. All remaining exons were sequenced using corresponding primers (table 1). Sequencing reactions were performed using the following conditions:

Table 1

Primers Used for Mutation Analysis

Exon	Primer Sequence		Size (bp)
	Forward	Reverse	
1	CCGCTTGAGACGCTCTAGTAT	CTGTCAGCACTTTCGGAGC	735
2	AATGGTGTCAGAGAGTTTACAG	ATAGCAAAAGTGGTCCTTGTTC	214
3	TTTATAGCAAAAGTGGTCCTTG	GAGGCTGGTGGGAAGTCTC	178
4	CCTTGCTGCTTCACCCTAG	CGTAGCAGCACATATAAAGCA	578
5	CTACATTTGTTCAACCATAACTG	CATCAACATCAAACCTCAAAATC	208
6	TCGTGTCAGATTCCCACCATA	ACGGATGTCTGACTTATGCCT	388
7	CATTCCCATGTATGAACGTCT	TCCTTCTTCCTGGGCTTACTAT	335
8	AGTGTCTTCAGCCTTTGTATTG	TGAGACAAGAAGAGATAGCAGAT	325
9	TAAAACCAACCTTCTTCATTAG	CATCGCTCCCATCATTGCT	245
10	GGCTTCTGGGGACTCACTG	CTACAGACACCACTTTTGAAGG	251
11	GTATCCACAAAGAGACCAGAAG	TAAGTCACTGTTGGTAGTTGGTG	448
12	GCTTGCCAGATACAGGAATC	CACCTGCCTAAACTTTCTGT	433
13	GAACACCTCATCCTCATTACCA	CCATGAATGTGTTTTGTCTCTT	316
14	GTTTCCGCTGTAAGGTAGTGT	CCACATAGTAAATGTTCCAG	266
15	TGCTAGTGGGTAGAGGTCAG	CATTCTAACCTGGCTTTCAGT	528
16	TGTGAAGACGGGATAACCTGA	TGCGGTATCAAGCCCTGTC	534
17	CCAGCCTTTGTGTAAGTCTAC	CTTCCAAACTTGCCCAGA	392
18	CGCTTTCTGCCTGTGACAT	GCATTGGCTGAAGGACAGAA	634
19	CACTTGATGGGCGTTCTGAG	GCATTGGCTGAAGGACAGAA	394
20	GGGTTCTCCAGCCAAAGACT	CAGAGCCTTCCAGCCCCACA	256
21	AAGAGGTAAGGGGCACAGC	GCAGAGGCAGGAGACTCAGA	313
22	GCTGAGTGTTGAGACCAGGA	GAGCAGCCGTCGTTTGTCT	252
23	GGGAGATGGTGCTGGCTAC	ATCTACCCATCACTAACCAGG	439
24	TTGATTTTGAGAGCATCTGGAC	CAGTGGGTCTAAGTGTCCGAG	860

95°C for 3 min, 98°C for 45 s, 50°C for 10 s, 60°C for 4 min, followed by 25 cycles of 98°C for 15 s, 50°C for 10 s, 60°C for 4 min.

Sequencing reactions were subsequently purified using a 96-well Sephadex plate (preparation of Sephadex G-50 containing microtiter filter plates from the University of Oklahoma Advanced Center for Genome Technology) and were dried in a Speedvac (Savant). Samples were rehydrated and denatured in 10 μl Hi-Di formamide loading buffer at 95°C for 3 min. Samples were electrophoresed on a 3700 DNA Analyzer (Applied Biosystems), according to the manufacturer's protocols.

Statistical Analyses

Hardy-Weinberg equilibrium (HWE) tests for all markers and LD tests between all pairs of markers were performed using independent individuals of HPC families and all sporadic case subjects and non-prostate cancer control subjects (GDA software, Weir et al. 1996). The HWE tests were based on exact tests, in which a large number of the possible arrays are generated by permuting the alleles among genotypes, and the proportion of these permuted genotypic arrays that have a smaller conditional probability than the original data is calculated. The LD tests were based on an exact test, assuming multinominal probability of the multilocus genotype, conditional on the single-locus genotype (Zaykin et al. 1995). A Monte Carlo simulation was used to assess the significance, by permuting the single-locus genotypes among individuals in the sample to simulate the null distribution. The empirical *P* values of both HWE and LD tests were based on 10,000 replicate samples. All six markers are in HWE in 159 probands with HPC. Both SNPs are in HWE in both case and control subjects.

Multipoint linkage analyses were performed using both parametric and nonparametric methods, implemented by GENEHUNTER-PLUS (Kruglyak et al. 1996; Kong and Cox 1997). Two genetic models were used for the parametric analyses. The autosomal dominant model was the same as that used by Smith et al. (1996). Under this model, the disease gene frequency of .003, incomplete penetrance, and phenocopies were assumed. Specifically, affected men were assumed to be disease-gene carriers with a fixed 15% phenocopy rate, and all unaffected men $\leq$75 years old and all women were assumed to be of unknown phenotype. In men >75 years old, the lifetime penetrance of gene carriers was estimated to be 63%, and the lifetime risk of prostate cancer for noncarriers was 16% in this age class. A similar autosomal recessive model was also used, except that the disease-gene frequency was set at .077, giving the same population disease frequency (Berry et al. 2000*b*). Linkage in the presence of heterogeneity was assessed by use of Smith's admixture test for heterogeneity (Ott 1998). In this test, two types of families are assumed: one type linked to the disease locus with a proportion

Table 2

Multipoint Linkage Results in 159 Families with HPC

Marker	Distance (cM)[a]	HLOD[b]		HLOD[b] (Recessive Model)		Allele-Sharing LOD	
		Two Point	Multipoint	Two Point	Multipoint	Two Point	Multipoint
D17S786	10.9	0	0	0	0	0	0
Ser217Leu	15.5	0	0	0	0	0	0
Ala541Thr	15.5	.14	0	.13	0	.03	0
D17S799	15.9	0	0	0	0	.07	0
D17S1843	22.1	0	0	0	0	.05	0
D17S783	28	0	0	0	0	.01	0

[a] Distance from 17 pter.
[b] HLOD = LOD score, assuming locus heterogeneity.

of α and the other type not linked, with the proportion $1 - \alpha$. A maximum-likelihood approach was used to estimate the proportion of linked families (α), by maximization of the admixed LOD score.

For the nonparametric analysis, the estimated marker identical by descent (IBD) sharing of alleles for the various affected relative pairs was compared with its expected values under the null hypothesis of no linkage. A statistic Z_{all} in the program was used (Whittemore and Halpern 1994). Allele-sharing LOD scores were then calculated, using the computer program ASM (Kong and Cox 1997), on the basis of the statistic Z_{all} with equal weight assigned to all families.

Family-based association tests were performed for all six markers in the 159 families with HPC, using the software package FBAT (Laird et al. 2000). Unlike the classic transmission/disequilibrium test (TDT), which is limited to a specific pedigree structure (one genotyped proband and two genotyped parents per pedigree), the FBAT uses data from nuclear families, sibships, or a combination of the two to test for linkage and LD between traits and genotypes. The test for linkage is valid when multiple affected members in each pedigree are used, and the power to detect linkage is increased if there is an association. The test for association is valid if the empirical variance is used to account for correlation between transmissions in families when linkage is present. In brief, the FBAT determines an S statistic from the data, which is the linear combination of offspring genotypes and phenotypes. The distribution of the S statistic is generated by treating the offspring genotype data as random and conditioning on the phenotypes and parental genotypes. When the marker is biallelic, a Z statistic (together with its corresponding P value) is calculated. When the marker is multiallelic, a χ^2 test is performed, with the number of df being equal to the number of alleles.

Population-based association tests were performed for the two polymorphisms in patients with prostate cancer and in control subjects without prostate cancer. An unconditional logistic regression is used to test for association between genotypes and affection status, adjusting for potential confounding variables such as age. The association tests were also performed only for white subjects to decrease the potential confounding effects of population stratification.

Results

Hardy-Weinberg Equilibrium and LD Tests

All four microsatellite markers and two SNPs are in HWE in this study population. The empirical P values for the HWE tests were .51, .48, .08, .52, .40, and .60 for D17S786, Ser217Leu, Ala541Thr, D17S799, D17S1843, and D17S783, respectively. There was a strong LD between the two SNPs ($P < 10^{-6}$), and almost all Thr541 allele carriers also carried Leu217. We observed three white patients with sporadic prostate cancer who had the haplotype Thr541 and Ser217.

Parametric and Nonparametric Linkage Analyses

There was no evidence for linkage between prostate cancer–susceptibility genes and markers at 17p13-17q11 in the 159 pedigrees with HPC (table 2). The total inheritance information in the 159 pedigrees using these six markers is intermediate (0.58–0.67). Parametric and nonparametric linkage analyses provided similar results. The multipoint LOD scores, under the assumption of heterogeneity and under either the dominant or the recessive model, were 0 across the region. Sixteen pedigrees had LOD scores (under the dominant model) ≥ 0.3, and three pedigrees had LOD scores ≥ 0.6. The highest LOD score, 0.97, occurred in a pedigree with six affected members (two affected siblings, an affected father, and three affected paternal uncles [father and one uncle without genotype]). The nonparametric allele-sharing LOD scores were 0 across the region.

The stratified linkage analyses did not provide evidence for linkage in any subsets of pedigrees (table 3). The allele-sharing LOD scores were 0 across the region regardless of age at diagnosis. In addition, allele-sharing LOD scores were 0 in pedigrees with three, four, and more than four affected members; in white and black

Table 3

Allele-Sharing LOD Scores in Subsets of Families with HPC

Family Characteristic	No. of Pedigrees	LOD Scores with					
		D17S786	Ser217Leu	Ala541Thr	D17S799	D17S1843	D17S783
Proband age at diagnosis:							
<65	79	.11	.02	.02	.03	.16	.19
≥65	80	0	0	0	0	0	0
No. of affected members:							
3	29	0	0	0	0	0	0
4	40	0	0	0	0	0	0
≥5	90	.03	.18	.14	.19	.21	.1
Race:							
White	133	0	0	0	0	.02	0
Black	14	0	0	0	0	0	0
Other	12	0	0	0	0	0	0
Male-to-male disease transmission:							
Yes	98	0	.01	0	.01	.02	.1
No	60	0	0	0	0	0	0
Probands with Leu217	89	0	0	0	0	0	0
Probands with Thr541	17	0	0	0	0	0	0

subjects; and in pedigrees with and without male-to-male disease transmission. When the linkage analyses were limited to the 89 pedigrees in which probands are Leu217 carriers or to the 17 pedigrees in which probands are Thr541 carriers, no evidence for linkage was found.

Mutational Screens for All Exons

To directly assess the HPC2/ELAC2 gene for mutations that may be segregating in the families with HPC reported here, the complete coding region of the gene was screened for sequence variants in 93 probands with HPC. Although the two previously reported missense changes were readily observed, no other DNA sequence variants were found that altered the amino acid sequence of HPC2/ELAC2.

Family and Population-Based Association Tests

Family-based linkage and association tests using a multiallelic method did not provide evidence for either linkage or LD between the markers and prostate cancer–susceptibility genes (table 4). The association tests using the biallelic method were also performed for the two single-nucleotide polymorphisms (SNPs). For the SNP Ser217Leu, 84 nuclear pedigrees were informative for the analysis. The observed S score was 187.0, and the expected S score was 194.3 (empirical variance 40.3; $Z = -1.15$; $P = .25$). For the SNP Ala541Thr, 24 nuclear pedigrees were informative for the analysis. The observed S score was 33.0, and the expected S score was 30.8 (variance 6.40; $Z = 0.87$; $P = .38$). The results were similar when the analyses were limited to white subjects.

Allele frequencies of the two SNPs were compared between patients with prostate cancer and control subjects. To decrease the confounding effect of racial differences, the comparison was limited to white subjects. The allele frequencies for Leu217 were 34.0%, 29.2%, and 27.2%, in the 134 probands with HPC, 228 patients with sporadic disease, and 182 unaffected control subjects, respectively. There was no statistically significant difference in the frequencies between the HPC case subjects and control subjects (Fisher's exact test [FET] $P = .08$), between the sporadic case subjects and control subjects (FET $P = .58$), and between all case subjects and control subjects (FET $P = .21$). The allele frequencies for Thr541 were 6.1%, 4.8%, and 4.4% in the probands with HPC, the patients with sporadic disease, and the unaffected control subjects, respectively. No significant difference was observed in the allele frequencies between the probands and control subjects (FET $P = .45$), between the patients with sporadic disease and the control subjects (FET $P = .87$), or between all patients and control subjects (FET $P = .65$).

Genotype frequencies of the two SNPs were also compared in an analysis restricted to white subjects (table 5). There was a trend toward higher Leu217 homozygous rates in the patients with HPC (11.2%) and in the patients with sporadic disease (8.3%) than in the control subjects (7.7%); however, the difference was not statistically significant. There was no statistical difference in the Thr541 carrier rates in the patients with HPC (10.5%), in the patients with sporadic disease (9.0%), or in the unaffected control subjects (9.0%). When the two SNPs are considered together, no significant difference in the frequencies was found. The frequencies of individuals carrying both Leu217 and Thr541 were 10.4% in the HPC case subjects, 8% in the sporadic case subjects, and 8.8% in the unaffected control subjects.

Table 4

Results of Family-Based Association Test in 159 Families with HPC

Marker	No. of Carriers	df[a]	χ^2	P
D17S786	11	6	2.4	.88
Ser217Leu	2	1	1.42	.23
Ala541Thr	2	1	.61	.44
D17S799	10	6	2.89	.82
D17S1843	14	6	4.91	.55
D17S783	10	7	6.11	.53

NOTE.—Families comprised 653 and 97 affected and unaffected subjects, respectively.

[a] Alleles observed in <10 subjects were not included in the analysis.

We also examined the relationships of Leu217 and Thr541 frequencies and Gleason scores and pathological stages in sporadic prostate cancer case subjects. There was no statistically significant difference in the genotypic frequencies of the two SNPs between the groups with low (≤6) and high (≥7) Gleason scores or between the groups with disease confined to the prostate and the group with non–organ-confined disease (table 6).

Discussion

We tested several alternative hypotheses in the current study. The first hypothesis—that HPC2/ELAC2 is a high-prevalence, high-penetrance major gene for prostate cancer—was rejected, because linkage results using both parametric and nonparametric methods in the 159 pedigrees with HPC did not provide any evidence for linkage. The finding of no novel mutations in the coding region of HPC2/ELAC2 in 93 probands with HPC is consistent with this conclusion. The lack of evidence for linkage from the parametric analyses (under either a dominant or recessive model) is unlikely to have resulted solely from the misspecification of the parameters in the genetic model. The impact of misspecification of penetrance estimates on the linkage results is small, as long as a dominant or recessive model is correctly specified (Clerget-Darpoux et al. 1986).

The second hypothesis—that the HPC2/ELAC2 was a less prevalent, high-penetrance major gene—was also rejected, because linkage evidence was not found when heterogeneity was assumed, which tested for a subset of pedigrees linked to this gene or region. No evidence for linkage was found in predefined subsets of families based on the pedigree characteristics, such as age at diagnosis, number of affected members per pedigree, male-to-male disease transmission, and race. Finally, no evidence for linkage was observed in subsets of pedigrees in which probands carried the Leu217 and/or Thr541 alleles.

The third alternative hypothesis—that the HPC2/ELAC2 is a common, low-penetrance modifier gene—was rejected, because neither family-based nor population-based tests found evidence for association between the genotypes at Ser217Leu and/or Ala541Thr and prostate cancer risk. Leu217 and/or Thr541 carrier rates in probands with HPC or in the patients with sporadic disease were not significantly increased, compared with unaffected control subjects.

Although these alternative hypotheses were rejected because of the absence of statistically significant differences, the results should be interpreted cautiously, be-

Table 5

Genotypes of Ser217Leu and Ala541Thr in Patients with HPC, Patients with Sporadic Disease, and Unaffected Control Subjects (White Only)

Ser217Leu	Ala541Thr	No. of Control Subjects	No. of Patients with SPC[b]	No. of Patients with HPC	OR[a] (95% CI) of SPC vs. Control Subjects	OR[a] (95% CI) of HPC vs. Control Subjects	OR[a] (95% CI) of All Patients vs. Control Subjects
Ser/Ser		97	114	58	1	1	1
Ser/Leu		71	95	61	1.14 (.75–1.71)	1.45 (.90–2.35)	1.39 (.98–1.97)
Leu/Leu		14	19	15	1.14 (.54–2.40)	1.63 (.71–3.73)	1.34 (.68–2.63)
Any Leu		85	114	76	1.14 (.77–1.68)	1.49 (.94–2.35)	1.26 (.87–1.84)
	Ala/Ala	166	211	111	1	1	1
	Ala/Thr	16	20	11	.98 (.49–1.96)	.95 (.42–2.15)	.99 (.52–1.87)
	Thr/Thr	0	1	2			
	Any Thr	16	20	13	1.03 (.52–2.04)	1.16 (.53–2.55)	1.09 (.58–2.05)
Ser/Ser	Ala/Ala	97	110	55	1	1	1
Any Leu	Ala/Ala	69	97	56	1.25 (.82–1.89)	1.43 (.87–2.35)	1.33 (.90–1.95)
Ser/Ser	Any Thr	0	3	0			
Any Leu	Any Thr	16	17	13	.94 (.45–1.95)	1.37 (.61–3.11)	1.10 (.57–2.14)

[a] All ORs were adjusted for age.

[b] SPC = sporadic prostate cancer.

cause various forms of genetic heterogeneity, high phenocopy rates, and incomplete penetrance in prostate cancer can significantly decrease the power to detect linkage and association of a true susceptibility gene. Different modes of inheritance have been reported for the transmission of prostate cancer in families, including autosomal dominant and X-linkage modes (Woolf 1960; Carter et al. 1992; Hayes et al. 1995; Monroe et al. 1995; Narod et al. 1995; Grönberg et al. 1997; Schaid et al. 1998; Cerhan et al. 1999; Schuurman et al. 1999), and various loci have been reported as prostate cancer–susceptibility genes, including HPC1 (MIM 601518; Smith et al. 1996; Cooney et al. 1997; Hsieh et al. 1997; McIndoe et al. 1997; Eeles et al. 1998; Neuhausen et al. 1999; Xu 2000), PCAP (MIM 602759; Berthon et al. 1998; Gibbs et al. 1999*a*; Whittemore et al. 1999; Berry et al. 2000*a*), HPCX (MIM 300147; Xu et al. 1998; Lange et al. 1999; Peters et al. 2001), CAPB (MIM 603688; Gibbs et al. 1999*b*; Berry et al. 2000*b*), and HPC20 (Berry et al. 2000*b*). With these various forms of genetic heterogeneity, it would not be surprising that only a small proportion of pedigrees and patients had prostate cancer that was attributable to the HPC2/ECLA2 gene. Furthermore, the high phenocopy rate caused by high prevalence of the disease can prevent the detection of linkage even in the pedigrees where the HPC2/ECLA2 gene segregates (e.g., some affected individuals in these pedigrees with HPC could be nongenetic case subjects) which lead to false recombinants in the linkage analysis and to misclassification in the association study. These problems could be compounded by incomplete and age-dependent penetrance of HPC2/ECLA2. Finally, some of the unaffected men could be HPC2/ECLA2 gene carriers but remain unaffected because of lack of background genes (modifier genes) and/or lack of environmental risk factors.

To investigate the power to detect linkage in the 159 pedigrees with HPC in the presence of genetic heterogeneity, high phenocopy rate, and incomplete penetrance, we performed a computer simulation study using FASTLINK. The dominant model, as described in the Families and Methods section, which incorporates a 15% phenocopy rate and 63% penetrance by age 75 years, was used to simulate a disease gene that segregates in the 159 pedigrees with HPC. A marker with six equally frequent alleles was simulated to be linked to the disease gene at a recombination fraction (θ) of .025, using these exact pedigree structures, affection status, and availability of DNA. When 20% of the 159 pedigrees were linked to the disease gene, 46%, 17%, and 6% of the 1,000 replicates reached allele-sharing LOD scores of 1, 2, and 3, respectively. When one-third of the 159 pedigrees were linked to the disease gene, 89%, 65%, and 40% times among the 1,000 replicates reached allele-sharing LOD scores of 1, 2, or 3, respectively. The simulation results suggested that we had reasonable power to reach suggestive evidence for linkage in our study sample only if one-third of the pedigrees segregate the gene. When the proportion of pedigrees that segregate the gene is below that level, the power is very limited. Clearly, a much larger collection of pedigrees with HPC is needed to detect linkage of genes that segregate in a small proportion of pedigrees.

Table 6

Genotypes of Ser217Leu and Ala541Thr in White Men with Sporadic Prostate Cancer

	No. (%) of Patients with			
	Gleason Score		Pathological Stage[a]	
Allele	≤6	≥7	OC	NOC
Ser217Leu:				
Ser/Ser	42	72	32 (45.07)	82 (52.23)
Ser/Leu	38	57	33 (46.48)	62 (39.49)
Leu/Leu	6 (6.98)	13 (9.15)	6 (8.45)	13 (8.28)
Ala541Thr:				
Ala/Ala	83	128	62 (87.30)	149 (92.55)
Ala/Thr	6 (6.74)	14 (9.79)	9 (12.68)	11 (6.83)
Thr/Thr	0 (0)	1 (.7)	0 (0)	1 (.62)

[a] OC = organ-confined disease; NOC = non–organ-confined disease.

Similarly, we estimated the power to detect an association in our study sample. When the point estimates of ORs and frequencies from Tavtigian et al. (2001) are used, the power to detect an OR of 2.4 at the significance level of .05, with a frequency of Leu217 homozygous carrier rate of 6.1% in control subjects, is 87% in our combined 364 patients and 182 control subjects. The power to detect an OR of 2.9 at the significance level of .05, with a frequency of any Thr541 carrier rate of 3.4% in control subjects, is 69% in our combined patient and control sample. However, if we consider the lower estimates of the 95% CI of the reported ORs, our study sample has very low power to detect this level of effect. For example, if the Leu217 and Thr541 have an OR of 1.3 each, our sample has only 18% and 13% power, respectively.

Considering the difficulties in the linkage and association studies of complex diseases and the lack of power to detect linkage and association of genes with relatively small effects, our negative linkage and association results are not surprising. On the basis of our linkage results, we probably can rule out any major gene that segregates in a large number of pedigrees, but we cannot rule out the possibility that a small proportion of our pedigrees segregate a major gene in the region. However, two pieces of evidence suggested that, even if there is a major gene in the region that segregates in a small number of pedigrees, it is unlikely that they are the Leu217 and/or Thr541 variants of the HPC2/

ECLA2 gene. The first piece of evidence comes from the negative linkage results in pedigrees whose probands carried the Leu217 and/or Thr541 alleles. If the variants of the Leu217 and/or Thr541 were high-penetrance mutations, we would expect to observe linkage in these pedigrees. Although substantial phenocopies in these pedigrees could disguise the linkage even if the variants were high-penetrance mutations, it is difficult to use this argument to explain the second piece of evidence that the Leu217 and/or Thr541 alleles are not overtransmitted to affected individuals in family-based association tests.

Because our case-control sample has a reasonable power to detect association when Leu217 homozygotes have an OR of 2.4 or when Thr541 carriers have an OR of 2.9, our negative association results suggested that the Leu217 and Thr541, separately or together, are not the modifier mutations that increase the prostate cancer risk at the previously reported magnitude in our study population. However, our results cannot rule out the association between these variants and prostate cancer, if these variants confer lower risks than the point estimates (in the lower ranges of the reported 95% CI). In fact, although the differences were not statistically significant, we observed higher homozygous Leu217 carrier rates in the patients (9.4%) than in the control subjects (7.7%) (OR = 1.3). It is interesting that these rates were highest in the patients with HPC (11.2%) (OR = 1.6), intermediate in the patients with sporadic disease (8.3%) (OR = 1.1), and lowest in the control subjects (7.7%).

Our study is the first reported replication study to investigate the linkage results at 17p11. The initial report by Tavtigian et al. (2001) found a maximum two-point LOD of 4.5 and a maximum three-point LOD of 4.3 in the 17p11 region in the first 33 pedigrees. They found a much weaker linkage in the additional 94 pedigrees. Several factors may explain the difference between their study and ours. Most of their pedigrees are large. The mean numbers of affected and genotyped affected members were 18.9 and 5.5 per pedigree, respectively. The mean numbers of affected and genotyped affected members were only 5.1 and 3.3, respectively, in our study. Interestingly, the most notable positive LOD scores in our study came from the 90 pedigrees with five or more affected members. The Utah pedigrees may be more homogeneous in both genetic and environmental background than our study pedigrees. The study by Tavtigian et al. mainly used two-point or three-point methods because of the large size of the pedigrees. These linkage methods are sensitive to allele frequencies, and false-positive linkage can arise when marker allele frequencies are wrongly assumed (Ott 1998). This is especially critical in the study of prostate cancer, because most parental genotype data are missing. Our linkage analyses were based on both two-point and multipoint analyses and thus were robust to the incorrect estimates of marker allele frequencies.

Our study is the second reported replication study to investigate the association between the two common HPC2/ELAC2 sequence variants and prostate cancer risk. For the Ser217Leu missense change, Tavtigian et al. (2001) found significantly higher homozygous Leu217 carriers in the related patients (13.3%) than in the unaffected related pedigree members (9.3%) or in the unrelated married-in unaffected males (6.1%). Rebbeck et al. (2000) did not report the homozygous Leu217 carrier rate in their study but found lower Leu217 carrier rates in the patients (30.8%) than in the control subjects (31.5%). We report here a higher Leu217 homozygous carrier rate in probands with HPC (11.2%) and in the patients with sporadic disease (8.3%) than in the control subjects (7.7%), although this difference is not statistically significant. For the Ala541Thr variant, Tavtigian et al. (2001) found a significantly higher Thr541 carrier rate in the related patients (9.8%) than in the unrelated married-in unaffected men (3.4%). Rebbeck et al. (2000) reported a marginally significant, higher Thr541 carrier rate in the patients (7.5%) than in the 266 age- and race-matched control subjects (3.5%). However, the Thr541 carrier rate was 5.7% in their 383 control subjects. We found no difference in the Thr541 carrier rates in the patients with HPC (10.5%), in patients with sporadic disease (9.0%), or in unaffected control subjects (9.0%). As an additional control population, we genotyped 90 independent subjects (all whites) from one of our nonprostate cancer study populations (ages 45–65 years). Although the prostate cancer status was unknown for this population, it represents general population control subjects. In this population, we found a similar frequency for Thr541 carrier rate (11.1%) (J.X. and L.Z., unpublished data).

Although the differences between studies are unexplained, several of the following factors may contribute: First, the point estimates of the ORs in the study by Tavitigian et al. (2001) could be overestimated, because the case subjects were not independent; most of their study pedigrees are large, and if some of the pedigrees were linked to this chromosomal region and the affected individuals carried the variants, they could inflate the frequency of the variants in the case subjects. Second, the young age of some of the control men may lead to potential misclassification, thereby decreasing the power to detect association. Even though the age differences between case and control subjects were not statistically significant and the ORs were adjusted for age, some of the younger control subjects in the study reported here (40–50 years of age) may develop prostate cancer later. Third, population stratification may lead

to false-positive findings. Although this is unlikely, because race was matched in the two positive association studies, it is still possible that there are different genetic backgrounds between case and control subjects within the whites. The present study employed a family-based association test, which is robust to population stratification. Fourth, genotyping error is a potential problem in case-control studies. Although caution has been exercised and some genotypes were confirmed by multiple methods (Rebbeck et al. 2000), genotyping error in other subjects cannot be ruled out. It is worth noting that all the significant findings were marginal and that one misclassified genotype may change the results. To address this issue in our study, genotypes of the 159 HPC probands were confirmed by three independent laboratories.

Caution is warranted when interpreting and generalizing from the results of the present population-based association study. The case subjects collected in our study had early mean age of onset and thus may represent more hereditary case subjects. The potential bias could be two ways, either bias toward a significant finding if the HPC2/ELAC2 contributing to the susceptibility or bias against a significant finding if other competing major locus contributing to the susceptibility in these subjects. The control subjects in our study came from a prostate cancer screen population; the group therefore is likely to be at high risk (because of self selection). Although this could partially account for the higher frequency (compared with the studies of Tavtigian et al. [2001] and Rebbeck et al. [2000]) of the suspected alleles observed in our control subjects, we think the impact is limited for the following three reasons. First, all the control subjects were carefully examined and had normal DRE and PSA results. Thus, they are unlikely to be case subjects, at least at the time of examination. Second, very few of the control subjects have a positive family history. We collected extensive information on family history of the control subjects, and only six control subjects reported positive family history (defined as affected father and/or brothers) among 182 white control subjects. Furthermore, when we performed additional analysis with the six individuals excluded, the results were similar. Third, the frequency of the suspect alleles in 90 additional control subjects was similar to the screen control subjects.

In summary, the results of the study reported here are not consistent with a major role for HPC2/ELAC2 as a prostate cancer susceptibility gene. In addition, we find no significant evidence that the Leu217 or Thr541 variants of the HPC2/ELAC2 increase prostate cancer risk in our study population.

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Electronic-Database Information

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Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for prostate cancer [MIM 176807], HPC2 [MIM 605367], HPC1 [MIM 601518], PCaP [MIM 602759], PCBP/CAPB [MIM 603688], and HPCX [MIM 300147])
Stanford Denaturing High-Performance Liquid Chromatography, http://insertion.stanford.edu/melt.html
University of Oklahoma Advanced Center for Genome Technology, http://www.genome.ou.edu/

References

Berry R, Schaid DJ, Smith JR, French AJ, Schroeder JJ, McDonnell SK, Peterson BJ, Wang ZY, Carpten JD, Roberts SG, Tester DJ, Blute ML, Trent JM, Thibodeau SN (2000*a*) Linkage analyses at the chromosome 1 loci 1q24-25 (HPC1), 1q42.2-43 (PCAP), and 1p36 (CAPB) in families with hereditary prostate cancer. Am J Hum Genet 66:539–546

Berry R, Schroeder JJ, French AJ, McDonnell SK, Peterson BJ, Cunningham JM, Thibodeau SN, Schaid DJ (2000*b*) Evidence for a prostate cancer–susceptibility locus on chromosome 20. Am J Hum Genet 67:82–91

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wohr G, Latil A et al. (1998) Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43. Am J Hum Genet 62:1416–1424

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861–689

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC (1992) Mendalian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 89:3367–3371

Cerhan JR, Parker AS, Putnam SD, Chiu BC, Lynch CF, Cohen MB, Torner JC, Cantor KP (1999) Family history and prostate cancer risk in a population-based cohort of Iowa men. Cancer Epidemiol Biomarkers Prev 8:53–60

Clerget-Darpoux F, Bonaiti-Pellie C, Hochez J (1986) Effects

of misspecifying genetic parameters in lod score analysis. Biometrics 42:393–399

Cooney KA, McCarthy JD, Lange E, Huang L, Miesfeldt S, Monite JE, Oesterling JE, et al (1997) Prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. J Natl Cancer Inst 89:955–959

Cottingham Jr. RW, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Eeles RA, Durocher F, Edwards S, Teare D, Badzioch M, Hamoudi R, Gill S, et al. (1998) Linkage analysis of chromosome 1q markers in 136 prostate cancer families: The Cancer Research Campaign/British Prostate Group UK Familial Prostate Cancer Study Collaborators. Am J Hum Genet 62:653–658

Gibbs M, Chakrabarti L, Stanford JL, Goode EL, Kolb S, Schuster EF, Buckley VA, Shook M, Hood L, Jarvik GP, Ostrander EA (1999*a*) Analysis of chromosome 1q42.2-43 in 152 families with high risk of prostate cancer. Am J Hum Genet 64:1087–1095

Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L, Ostrander EA (1999*b*) Evidence for a rare prostate cancer–susceptibility locus at chromosome 1p36. Am J Hum Genet 64:776–787

Grönberg H, Damber L, Damber JE, Iselius L (1997) Segregation analysis of prostate cancer in Sweden: support for dominant inheritance. Am J Epidemiol 146:552–557

Hayes RB, Liff JM, Pottern LM, Greenberg RS, Schoenberg JB, Schwartz AG, Swanson GM, Silverman DT, Brown LM, Hoover RN (1995) Prostate cancer risk in US blacks and whites with a family history of cancer. Int J Cancer 60: 361–364

Hsieh CL, Oakley-Girvan I, Gallagher RP, Wu AH, Kolonel LN, Teh CZ, Halpern J, et al (1997) Re: prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. J Natl Cancer Inst 89:1893–1894

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Laird NM, Horvath S, Xu X (2000) Implementing a unified approach to family based test of association. Genet Epidemiol (Suppl 1) 19:S36–S42

Lange EM, Chen H, Brierley K, Perrone EE, Bock CH; Gillanders E, Ray ME, Cooney KA (1999) Linkage analysis of 153 prostate cancer families over a 30-cM region containing the putative susceptibility locus HPCX. Clin Cancer Res 5: 4013–4020

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

McIndoe RA, Stanford JL, Gibbs M, Jarvik GP, Brandzel S, Neal CL, Li S, et al (1997) Linkage analysis of 49 high-risk families does not support a common familial prostate cancer–susceptibility gene at 1q24-25. Am J Hum Genet 61: 347–353

Monroe KR, Yu MC, Kolonel LN, Coetzee GA, Wilkens LR, Ross RK, Henderson BE (1995) Evidence of an X-linked or recessive genetic component to prostate cancer risk. Nat Med 1:827–829

Narod SA, Dupont A, Cusan L, Diamond P, Gomez JL, Suburu R, Labrie F (1995) The impact of family history on early detection of prostate cancer. Nat Med 1:99–101

Neuhausen SL, Farnham JM, Kort E, Tavtigian SV, Skolnick MH, Cannon-Albright LA (1999) Prostate cancer susceptibility locus HPC1 in Utah high-risk pedigrees. Hum Mol Genet 8:2437–2442

Ott J (1999) Analysis of human genetic linkage, 3d ed. Johns Hopkins Press, Baltimore

Peters MA, Jarvik GP, Janer M, Chakrabarti L, Kolb S, Goode EL, Gibbs M, DuBois CC, Schuster EF, Hood L, Ostrander EA, Stanford JL (2001) Genetic linkage analysis of prostate cancer families to Xq27-28. Hum Hered 51:107–113

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ, Malkowicz SB (2000) Association of HPC2/ELAC2 genotypes and prostate cancer. Am J Hum Genet 67:1014–1019

Schaid DJ, McDonnell SK, Blute ML, Thibodeau SN (1998) Evidence for autosomal dominant inheritance of prostate cancer. Am J Hum Genet 62:1425–1438

Schuurman AG, Zeegers MP, Goldbohm RA, van den Brandt PA (1999) A case-cohort study on prostate cancer risk in relation to family history of prostate cancer. Epidemiology 10:192–195

Smith JR, Freije D, Carpten JD, Grönberg H, Xu J, Issacs SD, Brownstein MJ, Bova GS, et al. (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371–1374

Tavitigian SV, Simard J, Teng DHF, Abtin V, Baumgard M, Beck A, et al. (2001) A strong candidate prostate cancer predisposition gene at chromosome 17p. Nat Genet 27: 172–180

Weir BS (1996) Genetic data analysis. Vol 2. Methods for discrete population genetic data. Sinauer Associates, Sunderland, MA

Whittemore A, Halpern J (1994) A class of tests for linkage using affected pedigree members. Biometrics 50:118–127

Whittemore AS, Lin IG, Oakley-Girvan I, Gallagher RP, Halpern J, Kolonel LN, Wu AH, Hsieh CL (1999) No evidence of linkage for chromosome 1q42.2-43 in prostate cancer. Am J Hum Genet 65:254–256

Woolf CM (1960) An investigation of familial aspects of carcinoma of the prostate. Cancer 13:739–744

Xu J, Meyers DA, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, et al (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20: 175–179

Xu J (2000) Combined analysis of hereditary prostate cancer linkage to 1q24-25: results from 772 hereditary prostate cancer families from the International Consortium for Prostate Cancer Genetics. Am J Hum Genet 66:945–957

Zaykin D, Zhivotovsky L, Weir BS (1995) Exact tests for association between alleles at arbitrary numbers of loci. Genetica 96:169–178

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Linkage of prostate cancer susceptibility loci to chromosome 1

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Abstract Three prostate cancer susceptibility genes have been reported to be linked to different regions on chromosome 1: HPC1 at 1q24–25, PCAP at 1q42–43, and CAPB at 1p36. Replication studies analyzing each of these regions have yielded inconsistent results. To evaluate linkage across this chromosome systematically, we performed multipoint linkage analyses with 50 microsatellite markers spanning chromosome 1 in 159 hereditary prostate cancer families (HPC), including 79 families analyzed in the original report describing HPC1 linkage. The highest lod scores for the complete dataset of 159 families were observed at 1q24–25 at which the parametric lod score assuming heterogeneity (hlod) was 2.54 (P=0.0006) with an allele sharing lod of 2.34 (P=0.001) at marker D1S413, although only weak evidence was observed in the 80 families not previously analyzed for this region (hlod=0.44, P=0.14, and allele sharing lod=0.67, P=0.08). In the complete data set, the evidence for linkage across this region was very broad, with allele sharing lod scores greater than 0.5 extending approximately 100 cM from 1p13 to 1q32, possibly indicating the presence of multiple susceptibility genes. Elsewhere on chromosome 1, some evidence of linkage was observed at 1q42–43, with a peak allele sharing lod of 0.56 (P=0.11) and hlod of 0.24 (P=0.25) at D1S235. For analysis of the CAPB locus at 1p36, we focused on six HPC families in our collection with a history of primary brain cancer; four of these families had positive linkage results at 1p36, with a peak allele sharing lod of 0.61 (P=0.09) and hlod of 0.39 (P=0.16) at D1S407 in all six families. These results are consistent with the heterogeneous nature of hereditary prostate cancer, and the existence of multiple loci on chromosome 1 for this disease.

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Introduction

Three prostate cancer (MIM 176807) susceptibility loci have been reported to be linked to three different regions on chromosome 1 (Smith et al. 1996; Berthon et al. 1998; Gibbs et al. 1999). By studying 79 hereditary prostate cancer (HPC) families (defined as three or more prostate cancer patients in first-degree relatives) ascertained at Johns Hopkins Hospital and 12 HPC families ascertained in Sweden, Smith et al. (1996) reported the first prostate cancer linkage to markers at 1q24–25 (HPC1; MIM 601518). The peak two-point lod score was 3.65 at a recombination fraction (θ) of 0.18 with marker D1S2883. Multipoint analyses with various combinations of three consecutive markers were performed, and lod scores greater than 4 were observed. Significant evidence for locus heterogeneity was obtained by an admixture test with the proportion of linked families (α) estimated to be 34%. The maximum multipoint lod score under the assumption of heterogeneity was 5.43. Non-parametric analyses provided comparable results, with a peak multipoint NPL score of 4.71 (P=1E-5). The linkage was stronger in the subset of families with early age of onset (Grönberg et al. 1997) and in families with

evidence of male-to-male disease transmission (Xu et al. 1998; Xu and ICPCG 2000).

The results of analysis of HPC1 linkage by other research groups have been variable. Several independent studies corroborated linkage to HPC1. Cooney et al. (1997) reported a linkage study of 1q24–25 in 59 prostate cancer families, each with two or more affected individuals. The peak NPL score was 1.58 at D1S466 (P=0.057) in the total 59 families but was 1.72 (P=0.045) in the subset of 20 families that met the criteria for hereditary prostate cancer families (three or more affected individuals within one nuclear family, affected individuals in three successive generations, and/or clustering of two or more individuals affected <55 years). Hsieh et al. (1997) provided further evidence in support of HPC1. In 92 unrelated families having three or more affected individuals, the NPL score was 1.71 (P=0.046). The evidence for linkage was stronger in the 46 families with a mean age at diagnosis of less than 67 years. The NPL score was 2.04 (P=0.023). Neuhausen et al. (1999) presented positive evidence for linkage in 41 large HPC families ascertained in Utah. The peak two-point lod was 1.73 (P=0.005) in the total families and a two-point lod of 2.82 (P=0.0003) in early age of onset families. Finally, in a study of 144 HPC families collected at Mayo Clinic, Berry et al. (2000a) did not find evidence for linkage at HPC1 region in the total sample but established HPC1 linkage in a subset of 102 families with male-to-male disease transmission. The peak NPL score was 1.99 (P=0.03) at D1S212.

Four other groups, however, reported no significant evidence for linkage of HPC1 in their study populations. McIndoe et al. (1997) found no evidence for linkage in this region in 49 high-risk prostate cancer families, with either a parametric lod score approach assuming homogeneity or a non-parametric analysis. There was also no evidence for linkage in the 18 families with early age at diagnosis (<65 years). Linkage analysis was further extended to 150 HPC families in this study population, and the linkage to HPC1 was strongly rejected (Goode et al. 2000). Berthon et al. (1998) reported results of a genome-wide screen and specific results from the 1q24–25 region in 47 French and German families. For the three markers in the 1q24–25 region, they found negative two-point lod scores assuming a dominant model. Eeles et al. (1998) published a linkage study of 1q24–25 in 136 prostate cancer families ascertained in United Kingdom, Quebec, and Texas, 76 of which had three or more affected individuals. They found negative NPL scores in this region in the total sample but positive NPL scores in a subset of 35 families with four or more affected members. Suarez et al. (2000a) obtained no evidence for the HPC1 locus in their 230 multiplex sibships, although positive linkage results in the region were observed. The Zlr was 2.10 (P=0.018) at D1S2141 in sibships with positive family history and Zlr=2.72 (P=0.003) at D1S1677 in sibships with negative family history. Suarez et al. (2000b) reported further negative findings for HPC1 in their 45 new multiplex sibships and four expanded families.

To clarify the inconsistent replication results and to test for linkage in a larger data set, a combined analysis for six markers in the 1q24–25 regions was performed in 772 HPC families ascertained by members of the International Consortium for Prostate Cancer Genetics (ICPCG) from North America, Australia, Finland, Norway, Sweden, and the United Kingdom (Xu and ICPCG 2000). This group of families included the majority of families analyzed in the studies described above but did not include the original 91 families described by Smith et al. (1996) in which the original linkage to HPC1 was found. Overall, there was some evidence for linkage, with a peak parametric multipoint lod score assuming heterogeneity (hlod) of 1.40 (P=0.01) at D1S212. The estimated α was 6%. The evidence for linkage was stronger in families with male-to-male disease transmission. The peak hlod was 2.56 (P=0.0006), and an α of 11% was seen in the subset of 491 families with male-to-male disease transmission families, compared with hlod of 0 in the remaining 281 families. Within the male-to-male disease transmission families, the α increased with early mean age of diagnosis (<65, α=19%) and number of affected family members (≥5, α=15%). The highest α was observed for the 48 families that met all three criteria (peak hlod =2.25, P=0.001, α=29%). The results from non-parametric analyses were consistent with the parametric analysis, with a peak NPL score of 1.14 at D1S212 in the total 772 HPC families. The strongest evidence for linkage at this region was observed in the 491 families with male-to-male disease transmission, with a peak NPL of 2.3 (P=0.01).

These results support the finding of a prostate cancer susceptibility gene linked to 1q24–25.

The second HPC locus (PCaP; MIM 602759) on chromosome 1 was reported in the data from 47 French and German HPC families by using the combination of genome-wide screening and fine mapping (Berthon et al. 1998). This locus is located at 1q42–43, which is about 60 cM from HPC1. The maximum two-point lod score was 2.7 at marker D1S2785. The multipoint parametric analysis yielded an hlod of 2.2, and the non-parametric multipoint analysis yielded an NPL score of 3.1 (P=0.001). The estimated proportion of linked families was 50% in the sample. Furthermore, the evidence for linkage was stronger in a subset of nine families with early mean age of onset (<60 years), with hlod of 3.31 and NPL of 3.32 (P=0.001). However, replication studies of this locus have yielded inconsistent results in other study populations. Gibbs et al. (1999) analyzed 152 HPC families by using markers spanning a 20-cM region of 1q42–43 and did not find evidence for linkage to an HPC susceptibility locus. No evidence for linkage was found in early age of onset families. The most suggestive evidence for linkage was found in subset of 38 families with five and more affected members, with NPL of 1.2 (P=0.1). Whittemore et al. (1999) evaluated linkage to the region by using three markers in 97 HPC families. Negative lod scores and NPL scores were observed in the total sample and in 48 early age of onset families and 49 late age of onset families. Berry et al. (2000a) did not find evidence for linkage at six markers at 1q42–43 region in either the total 144 HPC families studied or in the subset of early age of onset families. However, they

found suggestive evidence for linkage in 21 families that met all three criteria: male-to-male disease transmission, family mean age of onset <66, and more than five affected members. The peak NPL score was 1.45 (P=0.08).

The third HPC susceptibility locus on chromosome 1 (PCBP/CAPB; MIM 603688; at 1p36) was reported by Gibbs et al. (1999) in families with prostate cancer and brain cancer. Based on the data from an initial genome-wide screen in 70 HPC families, evidence for linkage was observed at 1p36, with a multipoint hlod of 1.65 and NPL score of 2.13 (P=0.02). A fine mapping study was then performed in the region with additional markers and an additional 71 HPC families. Stronger evidence for linkage in the region was seen in a subset of 12 families with a history of prostate cancer and primary brain cancer. The overall two-point lod score was 3.22 at D1S507 in this subset. In the younger age of onset group of six HPC families (mean age at diagnosis <66 years), a maximum two-point lod of 3.65 at D1S407 was observed. The peak multipoint lod score assuming heterogeneity was 0.81 in the six families. No evidence for linkage was seen in either early or late age of onset families without a history of brain cancer. To replicate the finding in an independent study population, Berry et al. (2000a) studied 13 HPC families with prostate cancer and brain cancer and found no evidence for linkage. Both multipoint lod scores and NPL scores were negative in the region. Badzioch et al (2000) found evidence of linkage to CAPB in families with early onset prostate cancer, although no association with other cancers was seen.

Other prostate cancer linkages located outside of chromosome 1 have been reported. In a linkage analysis of combined data of 360 prostate cancer families from North America, Finland, and Sweden, Xu et al. (1998) reported evidence for a prostate cancer susceptibility locus on Xq27–28 (HPCX; MIM 300147), with a maximum two-point lod of 4.6 at DXS1113. Parametric and non-parametric multipoint analyses provided results consistent with the two-point analysis. Stratified analysis on the basis of consistency with an X-linked mode of inheritance revealed that 129 families without male-to-male disease transmission contributed disproportionately to the evidence of linkage to this region. The other prostate cancer susceptibility locus resided at chromosome 20q13 (HPC20). It was identified in 162 North American families with three or more members affected with prostate cancer (Berry et al. 2000b). The highest two-point lod score was 2.69 at D20S196, and the maximum multipoint NPL score was 3.02 (P=0.002) at D20S887. The evidence for linkage at this region was stronger in subsets of families with male-to-male disease transmission, with fewer than five family members affected with prostate cancer, and with later average age of diagnosis (≥66 years). Recently, several genome-wide scans in prostate cancer families have been reported that implicate a number of novel loci as harboring prostate cancer susceptibility loci (Gibbs et al. 2000; Suarez et al. 2000a; Witte et al. 2000).

In light of the three reported prostate cancer susceptibility loci on chromosome 1 and the inconsistent results from replication studies, we systematically evaluated the linkage of prostate cancer susceptibility loci to the three proposed regions on chromosome 1 by using a dense marker set spanning the entire chromosome. We studied 159 HPC families ascertained at Johns Hopkins Hospital, including 79 described previously by Smith et al. (1996). This study had the following three specific goals: (1) to test for linkage(s) of prostate cancer susceptibility loci across the complete length of chromosome 1, especially with regard to (a) the linkage at 1q24–25 in the subset of 80 new families and linkage in the complete family collection, (b) the linkage at 1q42–43 in the complete family collection, and (c) the linkage at 1p36 in 6 families with history of both prostate cancer and primary brain cancer; (2) to investigate the relationship of the three reported linkages on chromosome 1 to one another; and (3) to perform stratified analyses to explore characteristics of the families supporting these linkages in terms of male-to-male disease transmission, mean age of onset within a family, and number of affected members.

Methods

Family collection

All 159 HPC families were collected and studied at the Brady Urology Institute at Johns Hopkins Hospital (Baltimore, Md.). The first 79 HPC families had been included in the initial HPC1 report (Smith et al. 1996), and the remaining 80 families were recruited subsequently. Families were ascertained from three resources. Most of them were ascertained through referrals generated as a response to a letter by one of us (P.C.W.) to 8000 urologists throughout the country. The second source was identified from family history records of the patient population seen at Johns Hopkins Hospital for treatment of prostate cancer. The remaining families came from respondents to articles published in a variety of lay publications describing our prostate cancer family studies. Prostate cancer diagnosis was verified by medical records for each affected male studied. Age of diagnosis of prostate cancer was confirmed either through medical records or from two other independent sources. All individuals in this study gave full informed consent.

Families were defined as having male-to-male disease transmission when there was evidence of paternal disease transmission in the families, including the following: (1) affected father and affected sons; (2) prostate cancer cases on the paternal side of the family, with no evidence of affected relatives on the maternal side; or (3) prostate cancer cases on the maternal side of the family and male-to-male disease transmission on the maternal side. The remaining families were defined as non-male-to-male disease transmission families. They had either an unknown mode of inheritance (insufficient data to determine inheritance pattern) or were consistent with an X-linked mode of inheritance.

The family characteristics of the 159 HPC families are shown in Table 1. The subsequently collected 80 HPC families tended to be smaller and more heterogeneous in terms of race/ethnicities, compared with the first 79 HPC families. The classification of the number of affected family members was based on their medical history, and not all affected members had DNA samples. Fourteen and eleven of the families in the complete data set were African-American and Ashkenazi Jewish, respectively.

Genotyping and markers

Fifty microsatellite markers across chromosome 1 were genotyped and analyzed for the study. These markers were selected based on

Table 1 Characteristics of prostate cancer families

	All families	1st 79 families[a]	2nd 80 families
Mean age at onset (years)	64.3	65.1	63.5
Mean number of affected family members	5.1	5.3	4.9
Mean number of affected family members with DNA sample	3.3	3.8	2.9
Male-to-male disease transmission			
No. families with male-to-male disease transmission	99 (62%)	47 (59%)	52 (65%)
No. families without male-to-male disease transmission	60	32	38
Age of onset			
No. families age onset <65	79 (50%)	35 (44%)	44 (55%)
No. families age onset >= 65	80	44	36
No. families with >=5 affected members	90 (57%)	48 (61%)	42 (53%)
No. families with 4 affected members	40	23	17
No. families with 3 affected members	29	8	21
Race/ethnicity			
Caucasian	133 (84%)	74 (94%)	59 (75%)
African American	14	3	11
Others	12	2	10

[a]These families were included in the initial linkage report of HPC1 locus (Smith et al. 1996)

the following three criteria: (1) in the regions where linkages were reported (1p36, 1q24–25, and 1q42–43), polymorphic markers were selected with a resolution of approximately 2 cM; (2) the markers with the highest lod scores in each of the three initial reports were selected; and (3) in the regions in between these three reported linkages, markers were selected with a resolution about 10 cM. We performed multiplex polymerase chain reaction (PCR) with fluorescently labeled primers (either fam, hex, or ned), and the resulting PCR fragments were separated by using capillary electrophoresis in a ABI 3700 sequencer. The genotypes were scored by using ABI software (Genotyper). A modified version of the program Linkage Designer (http://dnalab-www.uia.ac.be/dnalab/ld.html) binned the alleles and checked inheritance. The output from Linkage Designer was then analyzed further for any inconsistencies by running LINKAGE software (Lathrop et al. 1984; Cottingham et al. 1993) without disease information. The Hardy-Weinberg equilibrium test was performed by using computer program GDA (Weir et al. 1996) for all markers as another check for the quality of genotype. The final check that was performed on the data was to run CRIMAP (Lander and Green 1987) to determine the order and length of the chromosomal map and to detect double recombinants. Marker allele frequencies were estimated from the independent individuals in the data set. Marker order and distances were based on the Marshfield genetic map (Table 2). In the cases where the markers were unavailable from a Marshfield map, the order and distances were estimated from the data by using CRIMAP. We chose the markers D1S489 and D1S552 as the boundaries of 1p36 region because these markers and markers in between had NPL scores of more than 1 in the original report (Gibbs et al. 1999). Markers D1S452 and D1S249 were chosen as the boundaries of 1q24–25 region because these markers and markers in between had NPL scores of more than 2 in the original report (Smith et al. 1996). For the boundaries of the 1q42–43 region, we chose markers D1S251 and D1S2842 as these markers flank the region of positive two-point lod scores in the original report (Berthon et al. 1998).

Statistical analyses

Multipoint linkage analyses were performed by using both parametric and non-parametric methods, implemented by the computer program GENEHUNTER-PLUS (Kruglyak et al. 1996; Kong and Cox 1997). For the parametric analysis, the same autosomal dominant model that had been used in many of the previous prostate linkage studies was assumed (Smith et al. 1996; Berthon et al. 1998). Under this model, the disease gene frequency of 0.003, incomplete penetrance, and phenocopies were assumed. Specifically, affected men were assumed to be disease gene carriers, with a fixed 15% phenocopy rate, whereas all unaffected men under 75 and all women were assumed to be of unknown phenotype. In men aged over 75 years, the lifetime penetrance of gene carriers was estimated to be 63%, and the lifetime risk of prostate cancer for non-carriers was 16% in this age class. Linkage in the presence of heterogeneity was assessed by use of Smith's admixture test for heterogeneity (Ott 1998). In this test, two types of families were assumed, one type linked to the disease locus with a proportion of α, and the other type is not linked with the proportion $1-\alpha$. A maximum likelihood approach was used to estimate the proportion of linked families (α), by maximizing the admixed lod score (hlod).

For the non-parametric analysis, the estimated marker identical by descent (IBD) sharing of alleles for the various affected relative pairs was compared with its expected values under the null hypothesis of no linkage. A statistic "Z-all" in the program was used (Whittemore and Halpern 1994). Allele sharing lod scores were then calculated based on the statistic "Z-all" and assigning equal weight to all families by using the computer program ASM (Kong and Cox 1997).

Both hlod and allele sharing lod can be converted to a χ^2 (χ^2=4.6×hlod). Although the true distribution of the χ^2 under null hypothesis of no linkage is unknown, especially in the situation of multipoint analysis, we assume that the distribution is a mixture of one that is degenerate at zero, and one that can be approximated by the distribution of the maximum of two independent χ^2 variables, each with 1 degree of freedom (Faraway 1993). *P*-values were thus calculated by $0.5*(1-(1-p_1)(1-p_1))$, where p_1 is the *P*-value of χ^2 with 1 degree of freedom.

Linkage analyses conditional on the linkage results at other locations were used in the current study for two purposes. First, for the chromosomal regions that are unlinked but located adjacent to one another (for example, 1q24–25 and 1q42–43), conditional analysis was used to explore whether the evidence for linkage in families linked at one region (the conditional locus) extended to the other region (the test locus). In this case, in the analysis of linkage data for the test locus, a weight of 1 was assigned to families with positive linkage scores at the conditional locus, and families with zero or negative linkage scores at this locus were assigned a weight of 0. Second, for the chromosomal regions that were completely unlinked, conditional analysis was used to explore the interaction of two regions of linkage, either assuming heterogeneity interaction (families linked to one region do not link to another region) or multiplicative interaction (families linked to one region tend to

Table 2 Marker information

Markers	Distance	Heterozygotsity
D1S489	30	0.88
D1S402	31.1	0.94
D1S407	33.9	0.88
D1S3669	37.1	0.91
D1S552	45.4	0.88
D1S1622	55.8	0.92
D1S3728	89.6	0.95
D1S1665	102.1	0.80
D1S1728	109.1	0.86
D1S1588	125.6	0.86
D1S223	133.9	0.73
D1S1631	137	0.91
D1S248	139.1	0.80
D1S2809	144.5	0.76
D1S534	151.5	0.92
D1S514	152	0.65
HSD3B2	152.5	0.74
D1S1653	164.7	0.88
D1S2707	169.1	0.83
D1S1677	176.2	0.89
D1S2799	183.8	0.92
D1S1619	188.9	0.89
D1S452	189.4	0.93
D1S218	192.1	0.94
D1S2659	192.7	0.90
D1S212	194.4	0.94
D1S2883	195.5	0.92
D1S466	198.9	0.93
D1S2818	199	0.92
D1S158	200.6	0.94
D1S191	201.6	0.91
D1S2848	201.7	0.93
D1S202	202.2	0.91
D1S238	203.3	0.94
D1S422	206	0.89
D1S2757	209.8	0.91
D1S413	213.1	0.93
D1S249	221.2	0.93
D1S425	231.7	0.90
D1S2141	234	0.93
D1S399	240.3	0.93
D1S549	240.4	0.89
D1S251	245.6	0.94
D1S235	255.2	0.92
D1S2678	256.9	0.89
D1S2670	263.6	0.92
D1S2785	266.9	0.92
D1S321	268.1	0.90
D1S304	268.2	0.80
D1S2842	274.2	0.90

linked to another region). For the multiplicative interaction, the same weighting scheme mentioned above was used. For the heterogeneity interaction, families were assigned a weight of 1 if they had negative linkage scores at the conditional locus and a weight of 0 if they had zero or positive linkage scores at this locus.

Results

Multipoint linkage analysis with 50 markers spanning chromosome 1

Fifty markers spanning chromosome 1 were genotyped in 159 HPC families, and the data analyzed using both a parametric model and a non-parametric allele-sharing approach. The lod score curves are shown in Fig. 1. The strongest evidence for linkage in the complete data set was observed at D1S413 at 1q25. Evidence for linkage extended from this marker almost 100 cM proximally, as far as 1p13. Additional smaller peaks were detected at D1S3728 at 1p32 and at D1S235 at 1q42.

Analysis of HPC1

The marker D1S413 is located in the region previously defined as HPC1 by Smith et al. (1996). The hlod was 2.54 (P=0.0006), with an estimated α of 17%, and the allele sharing lod was 2.34 (P=0.001) for this marker (Fig. 1). In the 80 new HPC families, the evidence for linkage at this region is substantially weaker (hlod=0.44, P=0.14, and allele sharing lod=0.67, P=0.08) when compared with results from the 79 families described in the original report of HPC1 linkage (hlod=3.05, P=0.0002, and allele sharing lod=3.09, P=0.0002). The evidence for linkage across the region was very broad, with allele sharing lod scores greater than 0.5 extending 100 cM, flanked by markers D1S514 at 1p13 and D1S2141 at 1q32.

Analysis of PCaP

There was evidence for linkage at 1q42–43, but this did not reach statistical significance. The highest allele sharing lod and hlod were 0.56 (P=0.11) and 0.24 (P=0.25) at D1S235, respectively (Fig. 1). This latter marker was at approximately 255 cM from 1pter, located at the proximal boundary of the initially reported PCaP region.

Analysis of CAPB

Although there was no evidence for linkage at 1p36 in the complete set of families (Fig. 1), four of the six families with a history of both prostate cancer and primary brain cancer had positive linkage scores at 1p36 (PCBP/CAPB). The highest allele sharing lod and hlod in the region were 0.61 (P=0.09) and 0.39 (P=0.16) at D1S407, respectively, in the six families. Lod scores at 1q24–25 and 1q42–43 for these six families were zero throughout these regions.

Analysis of 1p32

A linkage signal approximately 85 cM from 1pter was observed in this analysis. The hlod was 0.93 (P=0.04), and the

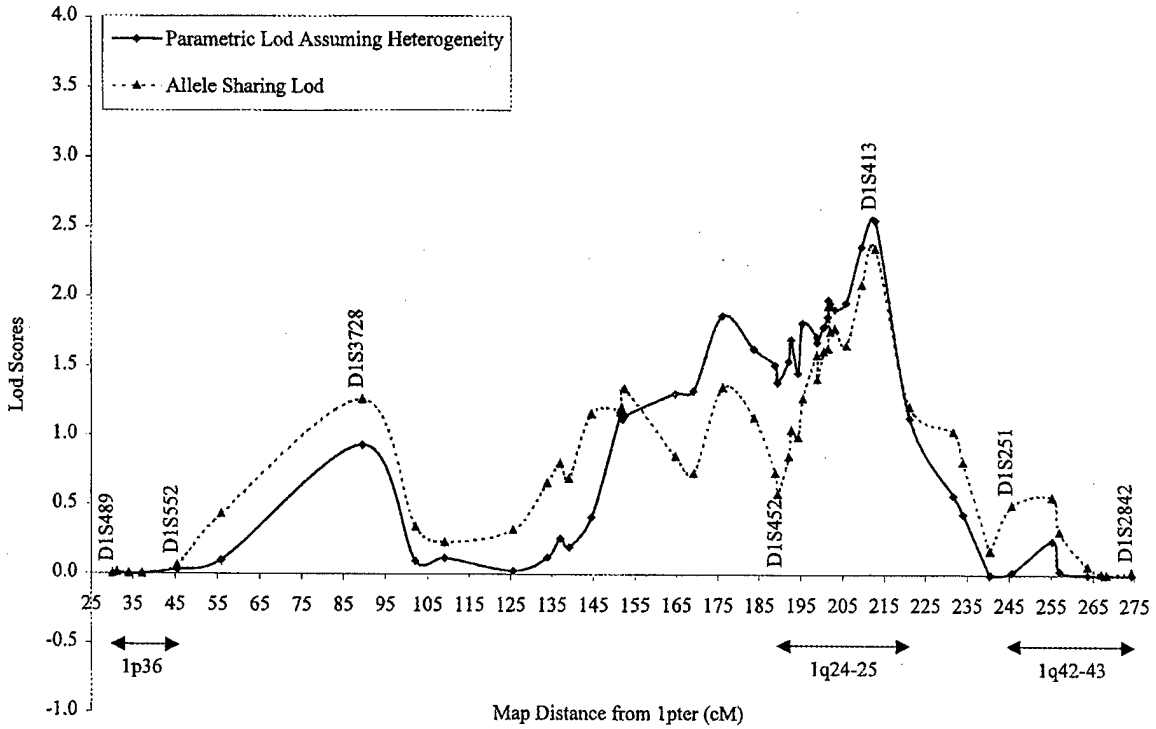


Fig. 1 Results of multipoint parametric and non-parametric linkage analyses of prostate cancer susceptibility loci by using 50 markers across chromosome 1 in 159 hereditary prostate cancer families (*solid line* parametric lod assuming heterogeneity, *dotted line* allele sharing lod, *diamonds* positions of markers, *circles* positions of markers)

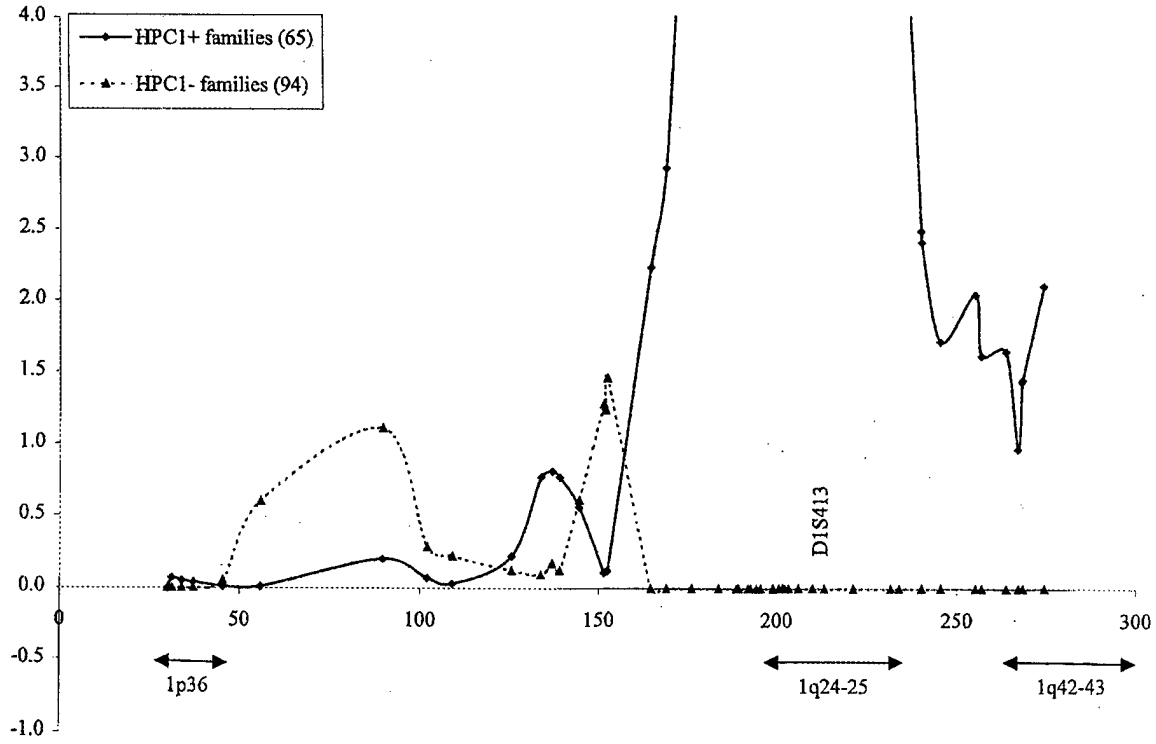


Fig. 2 Results of multipoint allele sharing lod conditional on the linkage result at D1S413 (*solid line* linkage results by assigning a weight of 1 or 0 for families that had allele sharing lod >0 or ≤0 at D1S413, respectively. *dotted line* linkage results by assigning a weight of 1 or 0 for families that had allele sharing lod <0 or ≥0 at D1S413, respectively)

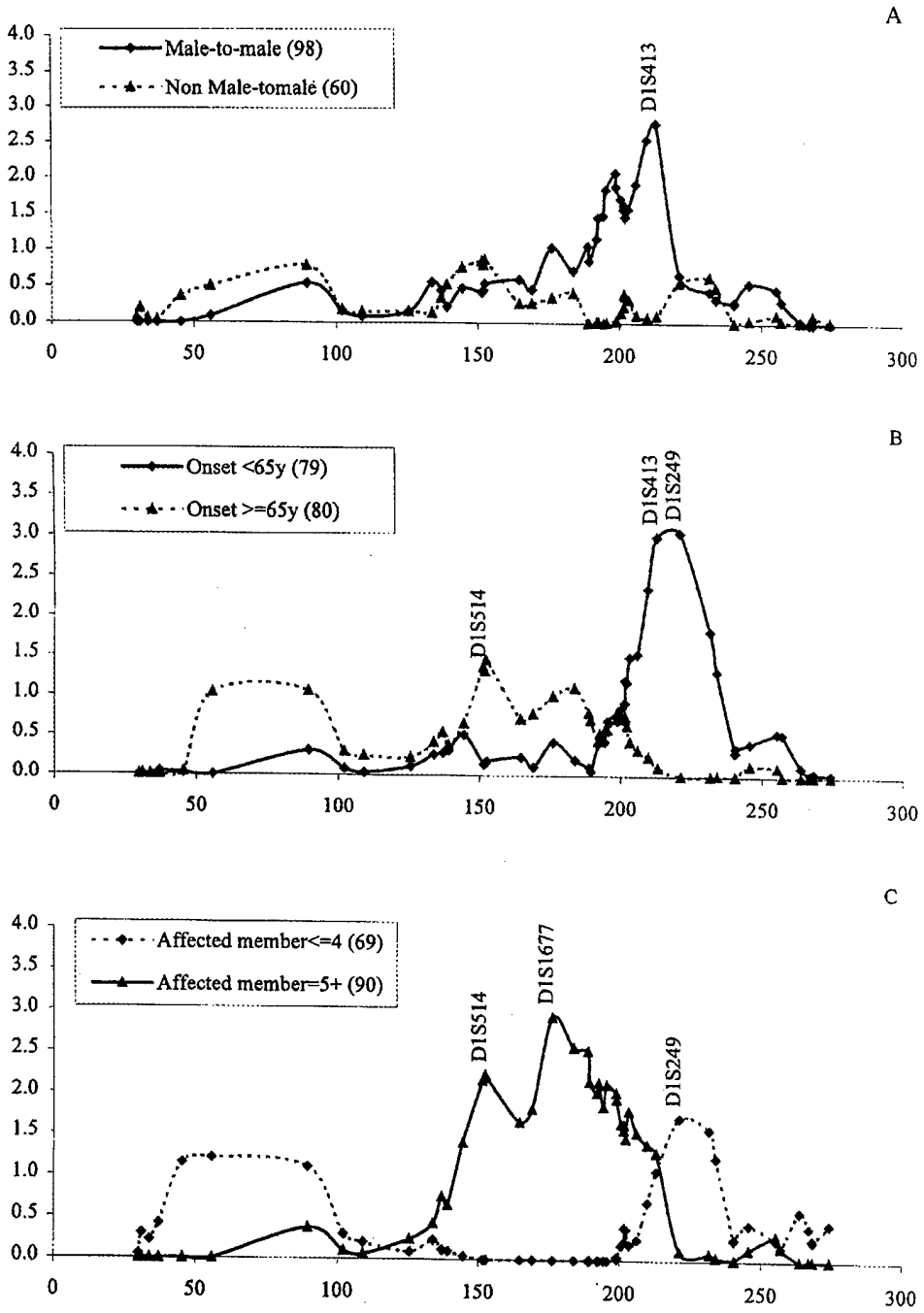


Fig. 3A–C Results of multipoint allele sharing lod for 50 markers on chromosome 1 stratified by the family characteristics. **A** Stratification by male-to-male disease transmission. **B** Stratification by family mean age of onset. **C** Stratification by number of affected family members

allele sharing lod was 1.26 (P=0.02) at marker D1S3728 at 1p32. However, these results should be considered preliminary until additional markers in the region are analyzed.

Relationship between linkage at 1q24–25 and other chromosome 1 loci

Since the strongest evidence for linkage was at 1q24–25, linkage data were re-analyzed for chromosome 1 markers conditional on the linkage information at 1q24–25 (Fig. 2). For 1q42–43, the evidence for linkage increased when families having a positive allele sharing lod at marker D1S413 at 1q24–25 region (n=65) were assigned a weight of 1 in the analysis, and the remaining families (n=94) were assigned a weight of 0. The allele sharing lod was 2.26 at D1S235 under these conditions, compared with 0.56 in the unconditional analysis. The results suggested that, in most families linked to 1q24–25, the evidence for linkage extended to markers in the 1q42–43 region. Testing for the independence of the allele sharing lod scores by family be-

tween the regions at D1S413 and D1S235 showed significant dependence between the two regions, with χ^2_1=17.27 (P=0.00003), again indicating that the families linked to 1q24–25 tended to be linked to 1q42–43, and vice versa. It is important to note that the largely inflated lod scores at 1q24–25 are artificial, since families linked to the region were assigned a weight of 1 and families unlinked to the 1q24–25 were assigned a weight of 0; therefore the value of the lod score for the region is not interpretable.

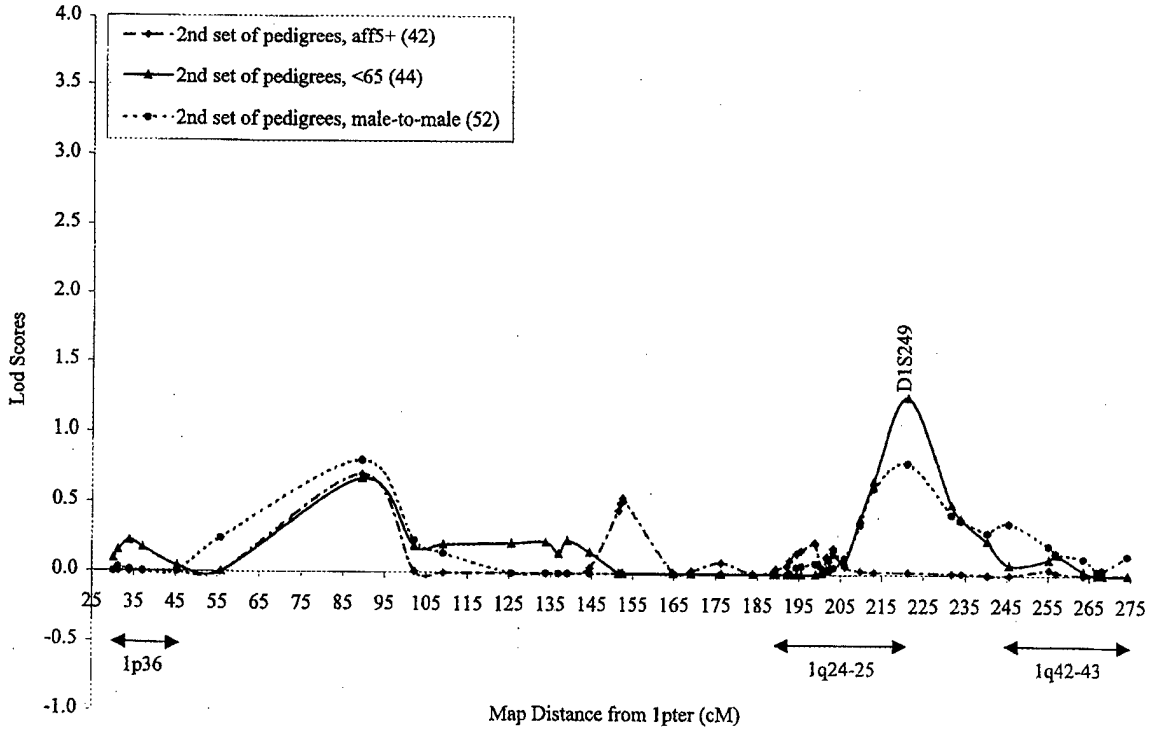
Conversely, when the 65 families that had positive allele sharing lod scores at marker D1S413 were assigned a weight of 0 and the remaining families were assigned a weight of 1, no evidence for linkage at 1q42–43 was observed, and hence little evidence for linkage at 1q42–43 in families not linked to 1q24–25. However, a linkage peak was observed approximately 155 cM from 1pter (1p13) under this conditional analysis (Fig. 2). The peak allele sharing lod was 1.46 (P=0.009) at D1S514. Testing for the independence of the allele sharing lod scores by family between the regions at D1S413 and D1S514 yield a χ^2_1=3.45 (P=0.06). These results suggested that the evidence for linkage at marker D1S514 and D1S413 came from different families.

Stratified analyses of linkage data based on family characteristics

Additional multipoint linkage analyses for all 50 markers across chromosome 1 were performed to include stratification of families based on the presence of male-to-male disease transmission, mean age of onset, and number of affected members (Fig. 3). Both parametric and non-parametric analyses gave similar results; thus, only the results of non-parametric analyses are shown. When the analyses were stratified by the presence or absence of male-to-male disease transmission, evidence for linkage was observed primarily at 1q24–25, occurring in the 98 families with male-to-male disease transmission. The peak lod score was 2.76 (P=0.0004) at D1S413. No statistically significant evidence for linkage was observed in any region in the remaining 60 families without male-to-male disease transmission (Fig. 3A). When families were divided by mean age of onset, the 79 families with early age of onset (<65) provided disproportional evidence for linkage at 1q24–25, with a peak lod of 3.05 (P=0.0002) between D1S413 and D1S249 (Fig. 3B). The 80 families with later age of onset had much weaker evidence for linkage in the region, with the peak lod of 1.45 (P=0.01) at D1S514. When families were stratified by the number of affected members, the 90 families with at least five affected members provided the strongest evidence for linkage in a broad region between 145 cM and 210 cM. The peak lod was 2.93 (P=0.0002) at D1S1677 (Fig. 3C). In the families with fewer than five affected members, evidence for linkage was weaker at D1S249. The peak lod was 1.71 (P=0.005).

The same stratification linkage analyses for the entire region on chromosome 1 were performed for the subset of the new 80 HPC families (Fig. 4). The 44 early age onset families provided the strongest evidence for linkage at the 1q24–25 region, with a peak allele sharing lod of 1.26 (P=0.02) at D1S249. The 52 male-to-male disease trans-

Fig. 4 Results of stratified multipoint allele sharing lod for 50 markers on chromosome 1 in the subset of 80 new HPC families (*aff5*+ five or more affected family members)



mission families provided disproportional evidence for linkage with allele sharing lod of 0.8 (P=0.05) at the same marker. Families with at least five affected members did not provide evidence for linkage at 1q24–25.

Discussion

Multipoint linkage analyses for prostate cancer susceptibility loci by using markers across chromosome 1 in 159 HPC families provided several findings of interest. First, the most significant evidence for linkage was observed at 1q24–25 in the complete data set, although the evidence for linkage from the subset of the 80 new families analyzed was weak. The evidence for linkage in this region spanned a broad interval, extending between 1p13 and 1q32. Second, a positive but not statistically significant linkage was observed at 1q42–43. Third, in six families with both prostate cancer and primary brain cancer patients, there was positive linkage at 1p36. Fourth, the evidence for linkage at 1q24–25 mainly came from a subset of families with male-to-male disease transmission and early age of onset.

Since 79 of the 159 families were included in the original report of linkage at 1q24–25 (Smith et al. 1996), the suggestive evidence for linkage at 1q24–25 in the current study cannot be interpreted as an independent confirmation but rather as a further evaluation of linkage in a larger sample. The independent confirmation of the linkage at the region, from the 80 new families, was weak with a peak hlod of 0.44 and an allele sharing lod of 0.67. The reasons for the different levels of support for the linkage in the initial 79 families and in the subsequent 80 families are unknown and could be attributable to a number of factors. (1) Most (70%) of the patients in the second cohort of families were diagnosed in 1992 or later and many of them through prostate-specific antigen (PSA) screening, whereas in the initial 79 families, only 46% were diagnosed by 1992 or later, and fewer were detected by PSA. The year and method of diagnosis could have an impact on the linkage results, probably by affecting the rates of phenocopies (Xu et al. 2000). (2) There are different degrees of genetic locus heterogeneity in the two sets of collected families.

In retrospect, it is possible that the proportion of families linked to 1q24–25 was over-estimated in the initial report (Smith et al. 1996); this is a common phenomenon in initial reports of linkage. Any linkage peak is likely to be at least the combination of two factors: the "true" evidence for linkage to a disease susceptibility gene in some families and the evidence for linkage observed attributable to the random variation by chance in other families (Suarez et al. 1994; Kruglyak et al. 1996). The random variation in favor of linkage may disappear in replication studies or, at the other extreme, result in decreased evidence for linkage. One approach to decrease the impact of random variation and to obtain a reliable estimate is to perform linkage in a large sample. This has been achieved in a combined data analysis of 1q24–25 from the ICPCG group (Xu and ICPCG 2000). The ICPCG study has replicated the linkage in an independent collection of 772 families and provided an estimate that 9% of HPC families are linked to 1q24–25 in the 863 HPC families that were available at the time (including the 79 HPC families and another 12 Swedish HPC families included in the initial finding by Smith et al. 1996).

The chromosomal region with evidence for linkage at 1q24–25 extends across a large genomic interval (~100 cM). The size of this region suggests the presence of multiple prostate cancer susceptibility genes in this interval. Preliminary evidence to support this possibility has been provided by the conditional linkage analyses and χ^2 tests, which indicate independence of the linkages to 1p13 and 1q24–25, i.e., different families are linked to different regions. The presence of multiple genes within this region could partially explain the difficulties experienced in the past 4 years by groups attempting to clone the HPC1 gene.

This is our first report of replication results of the linkage at 1q42–43 in this family collection. Although the linkage results at 1q42–43 are not statistically significant, our results are consistent with a prostate susceptibility locus (PCaP) in the 1q42–43 region. However, further studies with conditional analysis and the χ^2 test for the independence of lod score by families between the regions of 1q42–43 and 1q24–25 suggest that the evidence for both regions is related. A large fraction of families linked to 1q24–25 extend their linkage to 1q42–43. Regarding the previously reported characteristics of families linked to the PCAP locus (Berthon et al. 1997), the evidence for linkage was not increased in the 79 early age of onset families. The highest allele sharing lod was 0.53 (p=0.11) in this group.

This is also our first report of replication results of linkage at 1p36 in our family collection. With only six families with a history of both prostate cancer and primary brain cancer available for testing, we do not have an adequate sample size to make a reliable inference. However, the limited results from the current study are consistent with a prostate susceptibility locus (PCBP/CAPB) in the region. Two of the families had a mean age of onset of less than 65 years, and three of the families had five or more affected family members. In this small group, we have not observed a trend of increased evidence for linkage in the subset of early age of onset in these families, as indicated by the study of Gibbs et al. (1999).

There were a small number of African-American families (n=14) and Ashkenazi Jewish families (n=11) in our study sample. Both groups of families provided some evidence for linkage at 1q24–25, with allele sharing lod scores at D1S413 of 0.53 (P=0.11) and 0.70 (P=0.07), respectively. This compares with a lod score of 2.02 (P=0.002) at this marker for the 133 Caucasian families. Since racial differences in the marker allele frequencies are likely to exist between Caucasian and African-Americans, and linkage analysis is susceptible to the estimates of marker allele frequencies because of the missing parental data, we repeated the analysis for the African-American group by using the marker allele frequencies estimates from individuals in the 14 African-American families. The allele sharing lod was 0.49 at the same marker. No evidence for linkage at 1q42–43 or 1p36 was observed in the African-

American families. For the Ashkenazi Jewish families, allele sharing lod scores of 0.95 (P=0.04) at D1S3669 and 1.31 (P=0.014) at D1S2670 were observed in the CAPB and PCAP regions, respectively. The only Ashkenazi Jewish family with a history of both prostate and primary brain cancer gave a lod score of 0.29 in the CAPB region. Although these results are of interest, the small number of families in both these racial groups stresses the need for cautious interpretation of the data and for larger follow-up studies.

Prostate cancer is a complex disease with many factors that can potentially affect linkage studies. However, considering the public health significance of the disease, the continued evidence for an important role of genetic and familial factors (Carter et al. 1992; Lichtenstein et al. 2000) and the approaches available for mapping disease genes, e.g., the linkage-based positional cloning approach, represent important and potentially productive avenues for investigating and characterizing this common disease.

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Electronic-Database Information

- Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nml.nih.gov/Omim (prostate cancer, MIM 176807; HPC1, MIM 601518; PCaP, MIM 602759; PCBP/CAPB, MIM 603688; HPCX, MIM 300147
- GDA: Software for the Analysis of Discrete Genetic Data: http://lewis.eeb.uconn.edu/lewishome/gda.html
- Linkage Designer: http://dnalab-www.uia.ac.be/dnalab/ld.html

References

Badzioch M, Eeles R, Leblanc G, Foulkes WD, Giles G, Edwards S, Goldgar D, Hopper JL, Bishop DT, Moller P, Heimdal K, Easton D, Simard (2000) Suggestive evidence for a site specific prostate cancer gene on chromosome 1p36. The CRC/BPG UK Familial Prostate Cancer Study Coordinators and Collaborators. The EU Biomed Collaborators. J Med Genet 37:947–949

Berry R, Schaid DJ, Smith JR, French AJ, Schroeder JJ, McDonnell SK, Peterson BJ, Wang ZY, Carpten JD, Roberts SG, Tester DJ, Blute ML, Trent JM, Thibodeau SN (2000a) Linkage analyses at the chromosome 1 loci 1q24–25 (HPC1), 1q42.2–43 (PCAP), and 1p36 (CAPB) in families with hereditary prostate cancer. Am J Hum Genet 66:539–546

Berry R, Schroeder JJ, French AJ, McDonnell SK, Peterson BJ, Cunningham JM, Thibodeau SN, Schaid DJ (2000b) Evidence for a prostate cancer-susceptibility locus on chromosome 20. Am J Hum Genet 67:82–91

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wohr G, Latil A, et al (1998) Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2–43. Am J Hum Genet 62:1416–1424

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC (1992) Mendalian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 89:3367–3371

Cooney KA, McCarthy JD, Lange E, Huang L, Miesfeldt S, Monite JE, Oesterling JE, et al (1997) Prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. J Natl Cancer Inst 89:955–959

Cottingham RW Jr, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Eeles RA, Durocher F, Edwards S, Teare D, Badzioch M, Hamoudi R, Gill S, et al (1998) Linkage analysis of chromosome 1q markers in 136 prostate cancer families. The Cancer Research Campaign/British Prostate Group U.K. Familial Prostate Cancer Study Collaborators. Am J Hum Genet 62:653–658

Faraway JJ (1993) Distribution of the admixture test for the detection of linkage under heterogeneity. Genet Epidemiol 10:75–83

Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L, Ostrander EA (1999) Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. Am J Hum Genet 64:776–787

Gibbs M, Stanford JL, Jarvik GP, Janer M, Badzioch M, Peters MA, Goode EL, Kolb S, Chakrabarti L, Shook M, Basom R, Ostrander EA, Hood L (2000) A genomic scan of families with prostate cancer identifies multiple regions of interest. Am J Hum Genet 67:100–109

Goode EL, Stanford JL, Chakrabarti L, Gibbs M, Kolb S, McIndoe RA, Buckley VA, Schuster EF, Neal CL, Miller EL, Brandzel S, Hood L, Ostrander EA, Jarvik GP (2000) Linkage analysis of 150 high-risk prostate cancer families at 1q24–25. Genet Epidemiol 18:251–275

Grönberg H, Xu J, Smith JR, Carpten JD, Isaacs SD, Freije D, Bova GS, et al (1997) Early age of diagnosis in families providing evidence of linkage to the hereditary prostate cancer locus (HPC1) on chromosome 1. Cancer Res 57:4707–4709

Hsieh CL, Oakley-Girvan I, Gallagher RP, Wu AH, Kolonel LN, Teh CZ, Halpern J, et al (1997) Re: prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. J Natl Cancer Inst 89:1893–1894

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 84:2363–2367

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K (2000) Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343:78–85

McIndoe RA, Stanford JL, Gibbs M, Jarvik GP, Brandzel S, Neal CL, Li S, et al (1997) Linkage analysis of 49 high-risk families does not support a common familial prostate cancer-susceptibility gene at 1q24–25. Am J Hum Genet 61:347–353

Neuhausen SL, Farnham JM, Kort E, Tavtigian SV, Skolnick MH, Cannon-Albright LA (1999) Prostate cancer susceptibility locus HPC1 in Utah high-risk pedigrees. Hum Mol Genet 8:2437–2442

Ott J (1998) Analysis of human genetic linkage, 3rd edn. Johns Hopkins Press, Baltimore

Smith JR, Freije D, Carpten JD, Grönberg H, Xu J, Issacs SD, Brownstein MJ, Bova GS, et al (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371–1374

Suarez BK, Hampe CL, Van Eerdewegh P (1994) Problems of replicating linkage claims in psychiatry. In: Gershon ES, Cloninger CR (eds) Genetic approaches to mental disorders. American Psychiatric Press, Washington DC, pp 23–46

Suarez BK, Lin J, Burmester JK, Broman KW, Weber JL, Banerjee TK, Goddard KA, Witte JS, Elston RC, Catalona WJ (2000a) A genome screen of multiplex sibships with prostate cancer. Am J Hum Genet 66:933–944

Suarez BK, Lin J, Witte JS, Conti DV, Resnick MI, Klein EA, Burmester JK, Vaske DA, Banerjee TK, Catalona WJ (2000b) Replication linkage study for prostate cancer susceptibility genes. Prostate 45:106–114

Weir BS (1996) Genetic data analysis. II. Methods for discrete population genetic data. Sinauer Association, Sunderland, Mass.

Whittemore A, Halpern J (1994) A class of tests for linkage using affected pedigree members. Biometrics 50:118–127

Whittemore AS, Lin IG, Oakley-Girvan I, Gallagher RP, Halpern J, Kolonel LN, Wu AH, Hsieh CL (1999) No evidence of linkage for chromosome 1q42.2–43 in prostate cancer. Am J Hum Genet 65:254–256

Witte JS, Goddard KA, Conti DV, Elston RC, Lin J, Suarez BK, Broman KW, Burmester JK, Weber JL, Catalona WJ (2000) Genomewide scan for prostate cancer-aggressiveness loci. Am J Hum Genet 67:92–99

Xu J, ICPCG (2000) Combined analysis of hereditary prostate cancer linkage to 1q24–25: results from 772 hereditary prostate cancer families from the International Consortium for Prostate Cancer Genetics. Am J Hum Genet 66:945

Xu J, Meyers DA, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, et al (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20:175–179

Xu J, Isaacs SD, Wiley KE, et al (2000) Prostate cancer linkage to *HPC1*: effect of year of diagnosis. J Urol 163:55

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Original Investigation

Evidence for a prostate cancer linkage to chromosome 20 in 159 hereditary prostate cancer families

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Abstract. Prostate cancer is the most common malignancy diagnosed in men in the US. Genetic susceptibility to prostate cancer has been well documented. A region at chromosome 20q13 (HPC20) has been reported to be linked to a prostate cancer susceptibility gene. To confirm this finding, we genotyped 16 markers spanning ~95 cM on chromosome 20 in 159 hereditary prostate cancer (HPC) families. Positive (but not statistically significant) linkage scores were observed from 20pter to 20q11, with the highest non-parametric linkage (NPL) score for the complete dataset of 1.02 (P=0.15) being observed at D20S195 at 20q11. Evidence for linkage from parametric analyses with a dominant or a recessive model was weak. Interestingly, higher linkage scores were observed in the subsets of

families with a later age at diagnosis ($\geq$ 65 years; n=80, NPL=1.94, P=0.029 at D20S186), fewer than five affected family members (n=69, NPL=1.74, P=0.037 at D20S889), or without male-to-male disease transmission (n=60, NPL=1.01, P=0.15 at D20S117). The region with positive linkage scores spanned ~60 cM from 20pter to 20q11 in these subsets of families. Our results are consistent with a prostate cancer susceptibility locus on chromosome 20.

Introduction

Prostate cancer is of significant public health importance. With over 175,000 new cases being diagnosed in the US each year, prostate cancer causes a tremendous social and economic burden to patients, their families, and society (Landis et al. *1999*). The etiology of prostate cancer is unknown. Results from family studies, complex segregation analyses, and population-based studies consistently demonstrate genetic susceptibility to prostate cancer (Carter et al. *1992*; Schaid et al. *1998*). Several chromosomal regions that are likely to contain prostate cancer susceptibility genes have been identified in the past few years, including HPC1 at 1q24-25 (Smith et al. *1996*), PCAP at 1q42-43 (Berthon et al. *1998*), HPCX at Xq27-28 (Xu et al. *1998*), and CAPB at 1p36 (Gibbs et al. *1999*). Three genome-wide screens have identified many other regions with evidence for linkage (Smith et al. *1996*; Suarez et al. *2000*; Gibbs et al. *2000*; Witte et al. *2000*). Furthermore, a prostate cancer susceptibility gene, HPC2/ELAC2 on chromosome 17, has been identified by using a combined genome-wide screen, a fine mapping linkage study, and an association study (Tavtigian et al. *2001*). The association result has been replicated in an independent case-control study (Rebbeck et al. *2000*), although two other studies have not replicated the linkage and association findings (Xu et al. *2001a*; Vesprini et al. *2001*).

Recently, evidence for a new prostate cancer susceptibility locus at chromosome 20q13 (HPC20) was identified in 162 North American families with three or more members affected with prostate cancer (Berry et al. *2000*). The highest two-point LOD score was 2.69 at D20S196, and the maximum multipoint non-parametric linkage (NPL) score was 3.02 (P=0.002) at D20S887, ~3 cM from D20S196. The evidence for linkage at this region was stronger in subsets of 46 families without male-to-male disease transmission by using multipoint analyses (NPL=3.94, P=0.00007), 101 families with fewer than five family members affected with prostate cancer (NPL=3.22, P=0.0008), and 89 families with a later average age of diagnosis ($\geq$ 66 years, NPL=3.40, P=0.0006). The subset of 19 families with all three of these characteristics had an NPL of 3.69 (P=0.0001).

To examine prostate cancer linkage at 20q13, we used markers and analytical methods, similar to those described by Berry et al. (*2000*), in a study of 159 hereditary prostate cancer (HPC) families ascertained at the Johns Hopkins Hospital. Although no significant evidence for linkage at chromosome 20 was found in the complete 159 HPC families, elevated NPL scores were found in the complete set of families, and higher NPL scores were found in subsets of families with a later age of diagnosis ($\geq$ 65 years), fewer than five affected family members, or no male-to-male disease transmission.

Methods and materials

All 159 HPC families were collected and studied at the Brady Urology Institute at Johns Hopkins Hospital (Baltimore, Md.). Families were ascertained from three resources. Most of them were ascertained through referrals generated as a response to a letter by one of us (P.C.W.) to 8000

urologists throughout the country. The second source was the family history records of the patient population seen at Johns Hopkins Hospital for treatment of prostate cancer. The remaining families came from the respondents to articles published in a variety of lay publications describing our prostate cancer family studies. Prostate cancer diagnosis was verified by medical records for each affected male studied. All the 159 families had at least three affected family members, with the mean number of affected family members and affected family members genotyped being 5.1 and 3.3, respectively. There were 90 families with five or more affected family members, and 69 families with four or fewer affected family members. The age of diagnosis of prostate cancer was confirmed either through medical records or from two other independent sources. The mean age at diagnosis was 61.4 years. There were 79 and 80 families with mean age at diagnosis <65 years and $\geq$ 65 years, respectively.

The classification of male-to-male disease transmission was as defined elsewhere (Xu *2000*). There were 99 and 60 families with and without male-to-male disease transmission, respectively. The majority of the families were Caucasian (133). Fourteen families were African American.

Sixteen microsatellite markers spanning about 95 cM on chromosome 20 were genotyped. These markers were selected from Marshfield Comprehensive Human Genetic Maps (Broman et al. *1998*). Multiplex polymerase chain reaction (PCR) with fluorescently labeled primers (either fam, hex, or ned) was performed, and the resulting PCR fragments were separated by using capillary electrophoresis on an ABI 3700 sequencer. The marker order estimated from the data with CRIMAP (Lander and Green *1987*) was the same as that from the Marshfield database. The sex average marker distance (cumulative distance: 99 cM) estimated from the data was similar to that of the Marshfield database (95 cM) and was used for all the linkage analyses. Marker allele frequencies were estimated by using two different methods: either from the independent individuals or from all genotyped individual in the dataset. The multipoint linkage results from the two methods were remarkably similar, thus only the linkage results with respect to the marker allele frequencies estimated from independent individuals were reported.

Multipoint linkage analyses were performed by using both parametric and non-parametric methods, implemented by the computer program GENEHUNTER-PLUS version 1.2 (Kruglyak et al. *1996*; Kong and Cox *1997*). For the parametric analysis, the autosomal dominant and recessive models used in the study of Berry et al. (*2000*) were applied. Briefly, affected men had penetrances of 0.001 and 1.0 for non-carriers and carriers, respectively. The lifetime penetrances for unaffected men of age >75 years were 16% for non-carriers and 63% for carriers. Unaffected men of age $\leq$ 75 years and all women were not informative (i.e., unknown phenotype). Disease gene frequencies were 0.003 and 0.077, respectively, for the dominant and recessive model. Linkage in the presence of heterogeneity was assessed by use of Smith's admixture test for heterogeneity (Ott *1998*). A maximum likelihood approach was used to estimate the proportion of linked families (α), by maximizing the admixed lod score (hlod). A statistic (4.6 $\times$ hlod) was calculated that was approximately a mixture of χ^2 with 1 degree of freedom and a point mass at 0 (Faraway *1993*). *P*-values were thus calculated by $0.5*(1-(1-p_1)(1-p_1))$, where p_1 is the *P*-value of χ^2 with 1 degree of freedom. For the non-parametric analysis, the estimated marker identical by descent (IBD) sharing of alleles for the various affected relative pairs was compared with its expected values under the null hypothesis of no linkage. The S_{all} scoring function of the NPL scores (Whittemore and Halpern *1994*) was used. All the *P*-values of the NPL scores reported here were based on the exact distribution, as also reported in the program.

Results

Positive (but not statistically significant) linkage scores between a prostate cancer susceptibility locus and markers on chromosome 20 were observed in the complete 159 HPC families (Table 1). The highest multipoint NPL score was 1.02 (P=0.15), observed at D20S195 (~51 cM from 20pter). The highest multipoint HLOD was 0.08 at D20S889 under the dominant model and was 0.42 (P=0.15) at D20S107 (~56 cM from 20pter) under the recessive model. The markers D20S195 and D20S107 are at 20q11, ~20 cM proximal to the highest prostate cancer linkage region (20q13, ~73 cM from 20pter) reported by Berry et al. (*2000*). There was no evidence for linkage at 20q13 in our dataset. HLODs were zero, and the NPL scores were negative for multiple markers in the 20q13 region.

Table 1. Multipoint linkage results between the prostate cancer susceptibility locus and markers on chromosome 20 in 159 HPC families

	Markers at chromosome 20															
	S117	S889	S115	S186	S104	S112	S195	S107	S119	S178	S887	S196	S120	S100	S171	S173
Distance from 20 pter (cM)	2.8	11.2	21.1	32.2	37.5	39.1	50.7	55.6	61.6	66.0	72.1	74.8	83.3	84.6	95.5	97.9
Parametric analyses (HLOD)																
Dominant model	0	0.01	0	0.08	0.04	0.1	0.04	0	0	0.05	0	0	0	0	0	0
Recessive model	0.01	0	0.03	0.27	0.25	0.21	0.23	0.42	0	0.03	0	0	0	0	0	0
Non-parametric analyses (NPL)	0.42	0.78	0.59	0.97	0.77	0.81	1.02	0.80	0.55	0.45	-0.43	-0.40	0.22	0.01	-0.06	-0.52

Higher linkage scores were observed in the subsets of families that were likely to be linked to the HPC20 based on the study of Berry et al. (*2000*). Specifically, we found stronger evidence for linkage in subsets of families with a later age at diagnosis ($\geq$ 65 years), with four or fewer affected family members, or without male-to-male disease transmission (Tables 2, 3, 4). The positive linkage scores in these subsets spanned chromosome 20 from pter to q11 (~60 cM). Evidence for linkage was stronger in these subsets in non-parametric analyses. In the 80 families with mean age at diagnosis $\geq$ 65 years, the highest NPL scores were 1.94 (P=0.029) at D20S186, and 1.80 (P=0.038) at D20S112. The HLOD was 0.86 (P=0.046) at D20S112 based on the dominant model, and the HLOD was 0.94 (P=0.04) at D20S186 based on the recessive model. In the 69 families with four or fewer affected family members, the highest NPL scores were 1.74 (P=0.037) at D20S889 and 1.37 (P=0.08) at D20S195. Parametric analyses provided little evidence for linkage. In the 60 families without male-to-male disease transmission, the highest NPL scores were 1.01 (P=0.15) at D20S117 and 0.97 (P=0.16) at D20S112. In the 18 families having all three characteristics, there was no evidence for linkage from parametric and non-parametric analyses. The highest NPL score was 0.12 at 10 cM from pter in this subset of families.

Table 2. Multipoint non-parametric linkage analysis between the prostate cancer susceptibility locus and markers on chromosome 20 in subsets of HPC families

	Markers at chromosome 20															
	S117	S889	S115	S186	S104	S112	S195	S107	S119	S178	S887	S196	S120	S100	S171	S173
Distance from 20 pter (cM)	2.8	11.2	21.1	32.2	37.5	39.1	50.7	55.6	61.6	66.0	72.1	74.8	83.3	84.6	95.5	97.9
Mean age at diagnosis (years)																
<65 (n=79)	0.01	0.47	-0.02	-0.56	-0.54	-0.66	0.33	-0.03	-0.16	-0.29	-0.44	-0.52	-0.07	-0.15	0.18	-0.38
≥ 65 (n=80)	0.59	0.64	0.86	1.94	1.63	1.80	1.12	1.16	0.94	0.92	-0.18	-0.04	0.38	0.16	-0.26	-0.35
Number of affected family members																
≤ 4 (n=69)	1.34	1.74	0.93	0.78	0.93	0.86	1.37	1.20	1.06	0.62	0.15	-0.23	0.28	0.07	0.25	0
≥ 5 (n=90)	-0.58	-0.44	-0.02	0.63	0.23	0.35	0.15	0.06	-0.13	0.07	-0.55	-0.19	0.20	0.11	-0.14	-0.55
Male-to male disease transmission																
Yes (n=99)	-0.25	0.86	0.93	0.73	0.36	0.27	0.67	0.54	0.36	0.36	0.09	0.29	0.36	0.02	0.19	-0.21
No (n=60)	1.01	0.16	-0.23	0.64	0.78	0.97	0.80	0.61	0.44	0.27	-0.82	-1.01	-0.11	-0.01	-0.34	-0.57
Race																
Caucasian (n=133)	0.29	0.67	0.50	1.11	0.79	0.68	0.72	0.55	0.27	0.15	-0.76	-0.58	0.03	-0.06	-0.43	-0.75
African American (n=14)	-0.36	-0.61	-0.49	-0.14	-0.64	-0.23	-0.12	-0.25	-0.09	-0.08	-0.36	-0.53	0.11	-0.30	-0.32	-0.90

Table 3. Multipoint parametric linkage analysis between prostate cancer susceptibility locus and markers on chromosome 20 in subsets of HPC families (dominant model)

	Markers at chromosome 20															
	S117	S889	S115	S186	S104	S112	S195	S107	S119	S178	S887	S196	S120	S100	S171	S173
Distance from 20 pter (cM)	2.8	11.2	21.1	32.2	37.5	39.1	50.7	55.6	61.6	66.0	72.1	74.8	83.3	84.6	95.5	97.9
Mean age at diagnosis (years)																
<65 (n=79)	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0.01
≥ 65 (n=80)	0.01	0.00	0.06	0.81	0.63	0.86	0.30	0.25	0.15	0.52	0.02	0.04	0	0	0	0
Number of affected family members																
≤ 4 (n=69)	0.33	0.41	0.01	0	0	0	0.09	0.03	0.06	0	0	0	0	0	0.23	0.16
≥ 5 (n=90)	0	0	0	0.20	0.04	0.11	0	0	0	0.06	0	0	0	0	0	0
Male-to male disease transmission																
Yes (n=99)	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No (n=60)	0.11	0	0	0.14	0.16	0.20	0.13	0.03	0.05	0.30	0	0	0	0	0	0
Race																
Caucasian (n=133)	0	0.01	0	0.11	0.06	0.10	0.04	0	0	0.06	0	0	0	0	0	0
African American (n=14)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Multipoint parametric linkage analysis between prostate cancer susceptibility locus and markers on chromosome 20 in subsets of HPC families (recessive model)

	Markers at chromosome 20															
	S117	S889	S115	S186	S104	S112	S195	S107	S119	S178	S887	S196	S120	S100	S171	S173
Distance from 20 pter (cM)	2.8	11.2	21.1	32.2	37.5	39.1	50.7	55.6	61.6	66.0	72.1	74.8	83.3	84.6	95.5	97.9
Mean age at diagnosis (years)																
<65 (n=79)	0	0	0	0	0.01	0	0.25	0.22	0	0	0	0	0.01	0.01	0	0
≥ 65 (n=80)	0.07	0.04	0.23	0.94	0.37	0.39	0.02	0.20	0.10	0.10	0	0	0	0	0.02	0
Number of affected family members																
≤ 4 (n=69)	0.21	0.10	0	0.01	0.14	0.10	0.43	0.36	0	0	0	0	0	0	0	0
≥ 5 (n=90)	0	0	0.13	0.33	0.13	0.12	0	0.09	0	0.11	0.10	0.21	0.16	0.13	0.02	0
Male-to male disease transmission																
Yes (n=99)	0	0.07	0.09	0.14	0.02	0.01	0.02	0.08	0	0.08	0.28	0.54	0.46	0.34	0.05	0
No (n=60)	0.15	0	0	0.14	0.24	0.22	0.21	0.34	0.01	0	0	0	0	0	0	0
Race																
Caucasian (n=133)	0	0	0.02	0.26	0.24	0.16	0.16	0.36	0	0	0	0	0	0	0	0
African American (n=14)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The evidence for linkage at chromosome 20 came primarily from the 133 Caucasian families in our study population. The highest NPL score was 1.11 (P=0.13) at D20S186 in these families, whereas for the 14 African American families studied, the NPL scores were all zero.

Discussion

In an attempt to confirm the prostate cancer linkage at 20q13 (Berry et al. *2000*), we performed a linkage study for a prostate cancer susceptibility locus and markers on chromosome 20. Positive (but not statistically significant) linkage scores were observed from 20pter to 20q11. Interestingly, higher linkage scores were observed in the subsets of families with four or fewer affected family members, a

later age at diagnosis ($\geq$ 65 years), or without male-to-male disease transmission. Based on a simulation study that utilized the exact family structure and availability of DNA samples in our study, we estimated the empirical significance for the NPL of 1.02 in the complete 159 HPC families to be P=0.11. These results were consistent with a prostate cancer susceptibility locus at chromosome 20.

The region with the highest evidence for linkage at chromosome 20 was observed from 20pter to 20q11, not overlapping with the 20q13 region observed by Berry et al. (*2000*). Two comments may be relevant regarding this discrepancy. First, although the strongest evidence for linkage in the report of Berry et al. (*2000*) was at 20q13, the evidence for linkage extended proximally to 20p12. For example, the NPL score was approximately 1.5 at D20S186 (20p12) in the complete 162 families in the study of Berry et al. (*2000*). Second, factors including genetic heterogeneity, phenocopies, and incomplete penetrance may lead to false recombinants and push the linkage peak away from the true location of the disease gene (Lander and Schork *1994*). Since it is likely that these factors are present in familial prostate cancer, it is possible that the two different regions are, indeed, indicative of the same disease gene.

The prostate cancer linkage results at chromosome 20 from our study are weaker than those of Berry et al. (*2000*). Although the two studies are similar in two main aspects (i.e., the families were ascertained from North America, and all families have at least three affected family members), there are at least two differences. First, 9% and 7.5% of the families are African American and of other ethnic backgrounds, respectively, in our study, whereas all the families in the study of Berry et al. (*2000*) were Caucasian. The 14 African American families in our study did not provide any evidence for linkage at 20q. This group of families provided evidence for linkage at 1q24-25 (Xu et al. *2001b*). Second, the number of families having four affected family members was smaller in our study (43%) compared with the study of Berry et al. (*2000*; 62%). These differences, however, are unlikely to account for the different results between these studies for the following reasons. Restriction of the analysis to Caucasians only did not significantly increase the overall evidence for linkage, and the subset of 18 families that met all three criteria ($\geq$ 65 years, with $\leq$ 4 affected family members, and without male-to-male disease transmission) did not provide evidence for linkage at 20q13. Only one of these families in our study is African American. Thus, at this point, the different study results are attributable to unknown factors, and linkage studies with a larger number of families are needed to resolve this issue.

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References

Berry R, Schroeder JJ, French AJ, McDonnell SK, Peterson BJ, Cunningham JM, Thibodeau SN, Schaid DJ (2000) Evidence for a prostate cancer-susceptibility locus on chromosome 20. Am J Hum Genet 67:82-91

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wohr G, Latil A, et al (1998) Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43. Am J Hum Genet 62:1416-1424

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861-689

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC (1992) Mendalian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 89:3367-3371

Faraway JJ (1993) Distribution of the admixture test for the detection of linkage under heterogeneity. Genet Epidemiol 10:75-83

Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L, Ostrander EA (1999) Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. Am J Hum Genet 64:776-787

Gibbs M, Stanford JL, Jarvik GP, Janer M, Badzioch M, Peters MA, Goode EL, Kolb S, Chakrabarti L, Shook M, Basom R, Ostrander EA, Hood L (2000) A genomic scan of families with prostate cancer identifies multiple regions of interest. Am J Hum Genet 67:100-109

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179-1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347-1363

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 84:2363-2367

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037-2048

Landis SH, Murray T, Bolden S, Wingo PA (1999) Cancer statistics, 1999. CA Cancer J Clin 49:8-31

Ott J (1998) Analysis of human genetic linkage, 3rd edn. Johns Hopkins University Press, Baltimore

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ, Malkowicz SB (2000) Association of HPC2/ELAC2 genotypes and prostate cancer. Am J Hum Genet 67:1014-1019

Schaid DJ, McDonnell SK, Blute ML, Thibodeau SN (1998) Evidence for autosomal dominant inheritance of prostate cancer. Am J Hum Genet 62:1425-1438

Smith JR, Freije D, Carpten JD, Grönberg H, Xu J, Issacs SD, Brownstein MJ, Bova GS, et al (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371-1374

Suarez BK, Lin J, Burmester JK, Broman KW, Weber JL, Banerjee TK, Goddard KA, Witte JS, Elston RC, Catalona WJ (2000) A genome screen of multiplex sibships with prostate cancer. Am J Hum Genet 66:933-944

Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, Carillo AR, Chen Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupte JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S, Rommens J, Cannon-Albright LA (2001) A candidate prostate cancer susceptibility gene at chromosome 17p. Nat

Genet 27:172-180

Vesprini D, Nam RK, Trachtenberg J, Jewett MA, Tavtigian SV, Emami M, Ho M, Toi A, Narod SA (2001) HPC2 variants and screen-detected prostate cancer. Am J Hum Genet 2001 68:912-917

Whittemore A, Halpern J (1994) A class of tests for linkage using affected pedigree members. Biometrics 50:118-127

Witte JS, Goddard KA, Conti DV, Elston RC, Lin J, Suarez BK, Broman KW, Burmester JK, Weber JL, Catalona WJ (2000) Genomewide scan for prostate cancer-aggressiveness loci. Am J Hum Genet 67:92-99

Xu J (2000) Combined analysis of hereditary prostate cancer linkage to 1q24-25: results from 772 hereditary prostate cancer families from the International Consortium for Prostate Cancer Genetics. Am J Hum Genet 66:945-957

Xu J, Meyers DA, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, et al (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20:175-179

Xu J, Zheng SL, Carpten JD, Nupponen NN, Robbins CM, Mestre J, Moses TY, Faith DA, Kelly BD, Isaacs SD, Wiley KE, Ewing CM, Bujnovszky P, Chang B-L, Bailey-Wilson J, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB (2001a) Evaluation of linkage and association of HPC2/ELAC2 in patients with familial or sporadic prostate cancer. Am J Hum Genet 68:901-911

Xu J, Zheng SL, Chang B-L, Smith JR, Carpten JD, Stine OC, Isaacs SD, Wiley KE, Henning L, Ewing CM, Bujnovszky P, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB (2001b) Linkage of prostate cancer susceptibility loci to chromosome 1. Hum Genet 108: (in press)